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Short Report

Reversible Inactivation and Excitation of Nucleus Raphe Magnus Can Modulate Tail Blood Flow of Male Wistar Rats in Response to Hypothermia

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ABSTRACT

Background: The nucleus raphe magnus (NRM) is involved in thermoregulatory processing. There is a correlation between changes in the firing rates of the cells in the NRM and the application of the peripheral thermal stimulus. **Introduction:** we examined the effect of reversible inactivation and excitation of NRM on mechanisms involved in tail blood flow (TBF) regulation in hypothermia. **Methods:** Hypothermia was induced in Male Wistar rats and cannula was implanted above the NRM. To evaluate the effect of nucleus inactivation on TBF, the amount of TBF was measured by Laser Doppler in hypothermic rats, before and after lidocaine microinjection into NRM. TBF was also measured after glutamate microinjection to assess the effect of nucleus excitation in hypothermic rats. **Results:** Results indicated that after dropping TBF by hypothermia, microinjection of lidocaine into NRM significantly decreased TBF from 54.43 ± 5.7 to 46.81 ± 3.4, whereas glutamate microinjection caused a significant increase from 44.194 ± 0.6 to 98 ± 10.0 **Conclusion:** These data suggest that NRM have thermoregulatory effect in response to hypothermia. *Iran. Biomed. J. 12 (4): 237-240, 2008*

Keywords: Nucleus raphe magnus (NRM), Lidocaine, Glutamate, Hypothermia

INTRODUCTION

The greatest proportion of the cells that responds to skin temperature is in the nucleus raphe magnus (NRM), whereas there are few neurons in the raphe dorsalis and pontis that are influenced [1]. Blood is diverted from the skin to other organs in hypothermia [2] and the loss of heat in rats is regulated by blood flow under sympathetic control, through elaborate system of arteriovenous anastomoses of tail [3, 4]. Skin vasomotor and sudomotor centers are all important in thermo-regulation and the raphe magnus controls the skin blood flow [5, 6]. Previous studies have shown that electrical stimulation of the mid to caudal raphe magnus elicited sweat secretion and skin blood flow rises in the forepaw pads of decerbrate cats [7]. Excitation of neurons in the raphe region can cause vasoconstriction in the cutaneous bed without greatly affecting arterial pressure and changing blood flow in the mesenteric bed [8, 9]. It has also been demonstrated that chemical stimulation of the rostral venterolateral medulla can reduce tail temperature in hyperthermic, anesthetized rats [10].

The importance of medullar raphe in the control of rat tail blood flow (TBF) has been confirmed by (i) enhancement to tail sympathetic nerve activity after chemically activation of raphe neurons through

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glutamate microinjections, (ii) detection of sympathetic premotor neurons in medullar raphe nuclei after tracer injections into artery of tail, and (iii), increase in the expression of Fos immunoreactivity, mainly in the raphe, during hypothermia [11-13].

The aim of this study is to investigate the inactivation and excitation effects of lidocaine and glutamate on thermoregulatory processes in NRM of the rats.

MATERIALS AND METHODS

All chemical reagents were purchased from Sigma Company (Sigma, NY, USA).

Animals. Male Wistar rats (n = 24, weighting 250-300 g) were obtained from the Pasteur Institute of Iran (Tehran) and kept in the individual cage with controlled 12 h light/dark cycle at 23 ± 1°C with free access to water and food for one week. The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and an endotracheal tube was inserted via a tracheostomy [14]. They were then placed in Kopf stereotaxic instrument and, to access to the NRM, a 22-gauge stainless steel guide cannula was implanted stereotaxically into the NRM with the anterior-posterior, mediolateral, and dorsoventral coordination at 10.52, 0.0, and 10.1 mm, respectively [15].

Laser Doppler flowmeter. After one week of recovery, rats were reanesthetized with urethane (l g/kg; i.p.) and a Laser Doppler probe (1.33 mm probe) was positioned at the tail cutaneous surface around the tail artery for skin blood flow measurement [6]. The Doppler probe was connected to a flowmeter and the analog signal was digitized with a Maclab recording system (Laser Blood Flow Monitor (MBF3), Moor Instruments England, Class 3A Laser product, Max power 1.7, BSEN 60825, 1991).

Hypothermia. Body temperature was maintained at 22°C by wrapping the body with a cooling pad, leaving the tail exposed to the room temperature. A thermocouple was placed 6 cm past the anal sphincter to record the body temperature. Tail blood flow was measured before and after cooling the body. The implanted cannula in NRM was connected to a Hamilton microsyring by a polyvinyl tube and infusions were given over to seconds by air pressure generated by a hand-held syringe while the pipette tip was positioned in the NRM.

Drug treatment and TBF evaluation. Since the drugs are solved in artificial cerebrospinal fluid (aCSF), one type of control injection was performed: a vehicle control injection into the NRM. So, in control group (n = 8) aCSF and in other groups, lidocaine (1 µl, 2%) and glutamate (1 µl, 78 pmol/60 nl) were injected [16, 17]. TBF was measured immediately before and after each injection and in the case of lidocaine measurement was continued to 10 min.

Histological confirmation. After completion of the experiment, the ink was injected through the cannula to confirm proper implantation of guide cannula and the rats were per fused with normal saline solution followed by 9% formaldehyde and 30% sucrose solutions. A section (40 µm) of the brainstem was stained with Evan's blue dye and proper placement of the pipette tip in the NRM was verified with histological sections under the microscope.

Statistical analysis. Data is represented as mean ± SEM of 8 male rats in each group. Paired student's *t*-test was performed to evaluate the differences in mean of TBF before and after treatment.

All the animal experiments were performed in accordance with the guideline of Hamadan University of Medical Sciences and Health Services, Hamadan, Iran (http://www. Umsha.ac.ir) for experimental animals.

RESULTS AND DISCUSSION

In the present study, injection of aCSF into the NRM had no significantly effect on TBF compared to pre-injection values ($P>0.05$). Following the injection of lidocaine, TBF decreased from 54.43 ± 5.7 to 46.81 ± 3.4 ($P<0.05$) and the significant effect of lidocaine was observed ten min after injection at the hypothermic temperature (22°C), as shown in Figure 1A.

Previous studies have shown that NRM is a necessary component of the central nervous system thermoregulatory control circuitry in rats [17] and mid to caudal raphe magnus/pallidus stimulation increases skin blood flow in the forepaw pads of

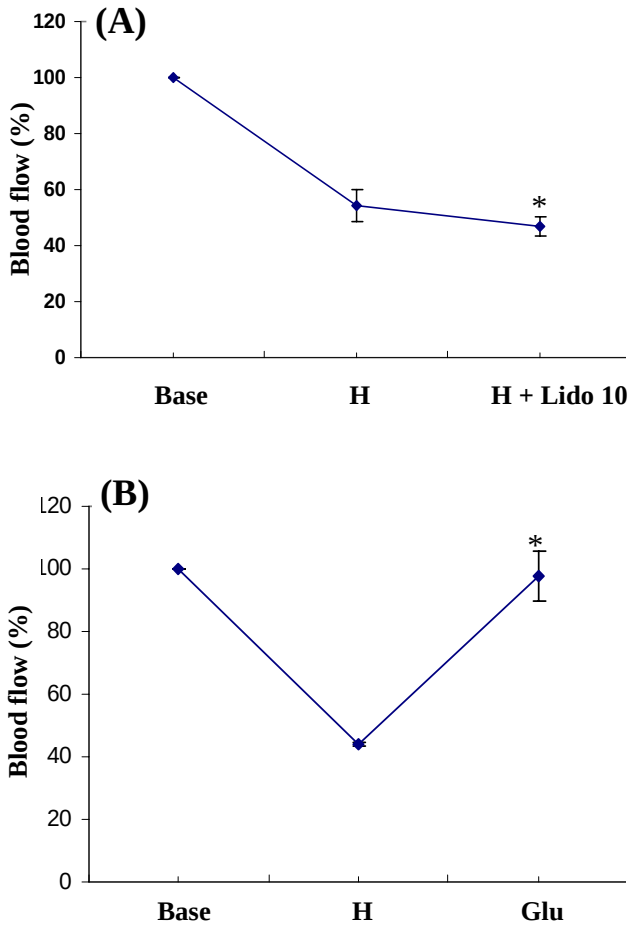

Fig. 1. Effect of unilateral injection of **(A)** lidocaine (1 µl, 2%) and **(B)** glutamate (78 pmol/60 nl) into the nucleus raphe magnus on tail blood flow in anesthetized cooled rats. Data are represented by mean ± SEM. Vertical bars denote SEM change from baseline values. Each bar represents the average data from 8 rats. Lido10 = ten min after lidocaine injection; H, hypothermia; Glu, glutamate injection; **P*<0.05.

decerbrate cats [8]. Therefore, NRM involves in the modulation of thermal information [15]. In this study, lidocaine, the fast-acting local anesthetic agent, was used to inactivate the NRM reversibly. Lidocaine blocks sodium channels, thus it inhibits neuronal electrical activity in the affected area. A 1.0 µl injection of 2% lidocaine solution has a 10-15 min effective period. This causes the lidocaineinfusion, an excellent technique for interrupting local neuronal activity without permanently altering the system [17]. In hypothermia, lidocaine injections into the medullary raphe were effective in suppressing the tail vasoconstriction, and therefore, lidocaine inhibits thermoregulatory effect of NRM and decreases TBF. Also, it shows another way to confirm previous results.

The investigation of the effect of glutamate injection on TBF showed a significant increase in TBF from 44.194 ± 0.6 to 98 ± 10, as indicated in Figure 1B ($P<0.05$). When tail blood flow was reduced by lowering the body temperature, the injection of glutamate restored the flow to levels generally observed in animals maintained at a higher baseline temperature. Therefore, according to our results, it can be concluded that chemical excitation of a subpopulation of neurons located in the raphe magnus region increases the blood flow of the tail cutaneous bed. Since glutamate, an excitatory neurotransmitter, can stimulate the NRM neurons, it may also decrease tail vasoconstriction in response to hypothermia. In addition, since sympathetic fibers play a key role in evoking vasoconstriction of the superficial vascular beds, it is suitable to propose that a reduction in the sympathetic outflow by centrally acting glutamate may be responsible for its hypothermic action in the NRM.

As hypothermia causes peripheral vaso-constriction, it is concluded that the vasoconstriction effect of hypothermia can be lowered by glutamate and augmented by lidocaine in NRM. Our findings add to evidence that the activation of the raphe magnus is involved in regulatory cutaneous blood flow in response to hypothermia in rats. In addition, our results highlight the importance of interaction between glutamate and thermoregulatory pathway in the nervous system. However, further investigations are required to evaluate NRM thermoregulatory mechanisms.

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