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Nitric oxide in the nucleus raphe magnus modulates cutaneous blood flow in rats during hypothermia

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ABSTRACT

Objective(s): Nucleus Raphe Magnus (NRM) that is involved in the regulation of body temperature contains nitric oxide (NO) synthase. Considering the effect of NO on skin blood flow control, in this study, we assessed its thermoregulatory role within the raphe magnus.
Materials and Methods: To this end, tail blood flow of male Wistar rats was measured by laser doppler following the induction of hypothermia.
Results: Intra-NRM injection of SNP (exogenous NO donor, 0.1- 0.2 µl, 0.2 nM) increased the blood flow. Similarly, unilateral microinjection of glutamate (0.1- 0.2 µl, 2.3 nM) into the nucleus increased the blood flow. This effect of L-glutamate was reduced by prior intra NRM administration of NO synthase inhibitor N^G-methyl-L-arginine or N^G-nitro-L-arginine methyl ester (L-NAME, 0.1 µl, 100 nM).
Conclusion: It is concluded that NO modulates the thermoregulatory response of NRM to hypothermia and may interact with excitatory amino acids in central skin blood flow regulation.

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Introduction

Nucleus Raphe Magnus (NRM) that possesses the greatest proportion of cells responding to cutaneous temperature (1) acts as a necessary component of the CNS thermoregulatory area in rats (2) and controls cutaneous blood flow (3, 4).There is some evidences demonstrating that the central area of the raphe is needed for the control of rat tail blood flow (2, 5). Following the injection of transsynaptic retrograde tracer into the wall of the tail artery, labeled sympathetic premotor neurons can be found in the medullary raphe nuclei (6). Neurons in the medullary raphe region constitute excitatory neurons that could be categorized as a class of sympathetic premotor neurons for the regulation of body temperature (7). Exposure of rats to cold temperature leads to an increase in Fos expression immunoreactivity, which is oncentrated n the raphe (8). In addition, excitation of neurons in the raphe region causes vasoconstriction in the skin blood vessels without profoundly influencing arterial pressure and change in the mesenteric bed flow (9, 10). This nucleus may not be involved in the thermal responses to CO_2 (11). Nitric oxide (NO) as a prominent second messenger in the central and peripheral nervous systems (12) participates in thermoregulation (4, 13, 14). Since NO acts as a central activator of heat defense mechanisms (15) and is synthesized in all mesensephalic raphe nuclei cells (16); therefore, in current study, we evaluated the role of NO on thermoregulatory action of NRM neurons.

Materials and Methods

All of the chemical agents used were purchased from Sigma Chemical Co (st. Lois, Mo, USA). In this study, 16 adult male Wistar rats (Pasteur institute of Iran, Tehran) weighing 250 and 300 g were used. All animal experiments were conducted according to the ethical guidelines set by the European Communities Council Directive. The rats were initially anesthetized by sodium pentobarbital (40 mg/kg, IP) (17).

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Following stereotaxically implantation of guide cannula (22- gauge stainless steel) into the NRM (AP: -10.52, ML: 0, DV: 10.1 mm) (18) and one week after recovery period, rats were reanaesthetized by urethane (l g/kg; IP) (8). A laser Doppler probe (1.33 mm) connected to a flowmeter, and the analog signal positioned on the tail cutaneous surface could measure tail blood flow (TBF) before and after cooling the body. Body temperature was monitored by a thermocouple placed 6 cm past the anal sphincter and maintained at the low temperature (27 ˚C) by a cooling pad wrapped around the body leaving the tail exposed to room air (6) All baseline data were collected for a minimum of 5 min after cooling. 0.1to 0.2 µl SNP (0.2 nmol) (19) was injected into the NRM by pressure through a 20– gauge stainless steel injection cannula attached to a 1-ml Hamilton microsyringe, which was mounted on a microdrive. Glutamate (0.1- 0.2 µl, 2.3 nM) (20) was administered before and after N^G-nitro-L-arginine methyl ester (L-NAME) microinjection (0.1 µl, 100 nM, n=8) (21). Each injection was made slowly over 10 min, and the micropipette was kept in place for additional 10 min after the injection. Postinjection data were then collected following every injection up to a maximum of 10 min. Since the drugs are solved in artificial cerebrospinal fluid (ACSF); therefore, one group of animals received ACSF by the same manner, and their data were considered as control.

For comparison of the excitation in the presence and absence of NO, we should add glutamate before and after the L-NAME injection to find the NO involvement addition to glutamate excitatory effect and so to reach two goals: contribution of NO by subtraction of glutamate (GLU) and GLU + L-NAME and contribution of glutamate alone that is GLU+ L-NAME value.

After the completion of the experiment procedures, immediately prior to sacrifice, ink was injected through the cannula. Then rats were perfused with saline and 9% formaldehyde, and finally with 30% sucrose solution. Brainstem sections (40 µm) were stained with cresyl violet, and proper placement of the needle tip in the NRM was also verified under the microscope (22). Animals with incorrect location of cannula were discarded from data analysis.

Data were expressed as means ± SEM. Tail blood flows were statistically analyzed by using one way ANOVA. *P*-value < 0.05 was used as the confidence level for all statistical tests.

Results

In Figure 1, location of raphe was demonstrated. Histological analysis showed that the location of cannula was correct in the NRM.

In this study, injection of ACSF into the NRM had no significant effect on TBF compare to pre-injection values in normothermic rats (*P*-value < 0.05). To determine the effect of NO increment in NRM during hypothermia, SNP was microinjected into the NRM in normothermic and hypothermic groups. In normothermic group, SNP increased the TBF from 96±3.1 to 138± 3.5 (*P*-value < 0.05) while in hypothermic group, SNP increased the TBF from 37.36±3.6 (basal level, during hypothermia) to 49.68±2.43 (*P*-value <0.05) (SNP±hypothermia) (Figure 2).

Unilateral microinjection of L-glutamate (2.3 nmol) into the NRM produced remarkable increment of blood flow from 44.199±0.6 (in cooled rats) to 98±8. After pretreatment with L-NAME (100 nmol) as NO synthase inhibitor for 10 min, the responses to L-glutamate were attenuated significantly (7±47.3 (*P*-value < 0.05)) (Figure 3). It means that L-NAME prevented the blood flow augmentation by glutamate during hypothermia. Rectal temperature and blood pressures were maintained within normal physiological limits.

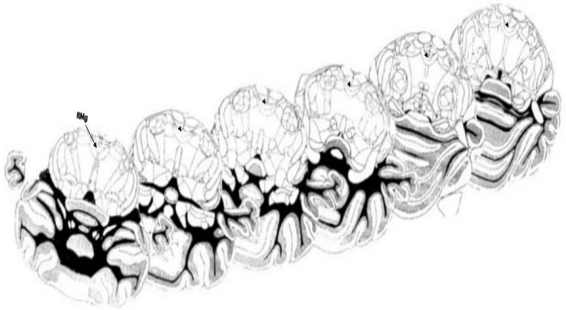

Figure1. Location of the cannulation in the nucleus raphe magnus. Arrowheads indicate the microinjection sites in these serial sections according to atlas of Paxinos. Maps and coordinates (from bregma) are taken from the atlas of Paxinos and Watson

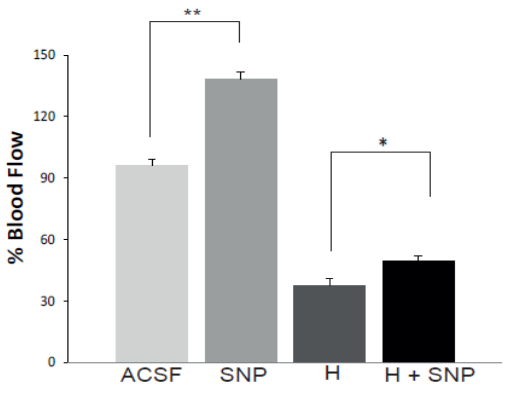

Figure 2. Effect of sodium nitroprusside injection into the nucleus raphe magnus on tail blood flow in anesthetized cooled rats. Data are represented by Mean± SEM. ACSF: Injection of ACSF into the NRM, H: Hypothermia, SNP: sodium nitroprusside injection; * *P*-value < 0.05 and ** *P*-value<0.01 n=8

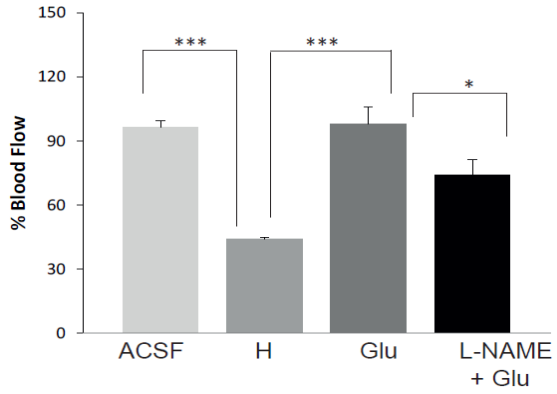

Figure 3. Effect of glutamate unilateral injection into the nucleus raphe magnus (2.3 nmol) before and after injection of nitric oxide synthase inhibitor on tail blood flow in anesthetized cooled rats. Data are represented by Mean±SEM. ACSF: Injection of ACSF into the NRM, H: Hypothermia, Glu: glutamate injection; * *P*-value< 0.05 and *** *P*-value<0.001, n=8. Each column represents the average value from 8 rats. Vertical bars represent SEM change from baseline values

Discussion

This study describes the role of NRM nitric oxide in modulating heat production.

Cutaneous blood flow brings metabolic heat to the surface of the body where it is in contact with the environment, especially from hairless skin areas, such as the rat tail (23).

In a study, it is found that caudal raphe nuclei (pallidus, magnus and obscures) as the principal serotonergic nuclei are involved in the body temperature regulation. They receive projections from the dorsomedial hypothalamus and project to spinal circuits that control the rat tail blood flow. The rat tail is a major organ of heat loss in this species. Multiple lines of evidence have implicated that these medullary serotonergic systems involve in both thermoregulatory warming responses and thermoregulatory cooling responses (24).

As the results show, when tail blood flow was diminished by reduction of the body temperature, glutamate microinjection restored the flow near to basal level generally observed in animals maintained at baseline temperature. Evidences show that electrical or chemical stimulation of the raphe can indeed affect the vasomotor supply to the rat's tail and the most effective raphe sites involving in tail vasoconstriction are placed more caudally than rostrally (2). Activation of raphe neurons by glutamate microinjection causes a great increase of tail sympathetic nerve activity (5), and premotor neurons located in the rostral medullary raphe control the tail circulation (25).

In this study, we found that intra NRM injection of SNP was effective in preventing thermal vasoconstriction of rat tail vessels in response to hypothermia, but inhibition of the NO pathway in the NRM by injection of L-NAME interferes with this excitatory effect of glutamate in the nucleus and then on tail blood flow. It has been shown that the stimulation of NMDA receptors increases formation of NO and resultant cGMP levels that can be prevented by NO synthase inhibitors. In addition, NO increases the release of excitatory amino acids by cGMP-dependent processes in the dorsomedial medulla (11). The interference of L-NAME with blood flow increment by glutamate in this study may be due to either decrement of NO and resultant cGMP or decrement the activity of NMDA receptors by NO.

In other study, L-NAME reduced NO facilitation of excitatory amino acid-evoked discharge during baroreceptor activity or cardiopulmonary afferent fibers activation in NTS neurons (26).

Current results are consistent with the hypothesis that central nitric oxidergic transmit-ssion plays an important role in the control of TBF adjustment during hypothermia, controlling heat production as shown by the augmented blood flow in SNP-treated rats.

These data agree with our previous study that intra NRM lidocaine injection was effective in suppressing the tail vasoconstriction in response to hypothermia and reconfirm the NRM functions in neuromodulation of thermal information.

Many studies implicated that intracerebro-ventricular (ICV) injection of NOS blockade (15, 27), or administration of NO donor (27), all demonstrate tonic activity of central sympathetic outflow by NO. Since sympathetic fibers play a key role in superficial vasoconstriction, it is satisfactory to suggest that the sympathetic outflow reduction by NO in the raphe may be responsible for its thermoregulatory action in the CNS (15, 28).

Conclusion

At the central level, the specificity of NO actions on physiological temperature regulation, mainly hypothermia was concluded. Our findings highlight the importance of interaction between NO and thermoregulatory pathway in the raphe magnus. Taken together, these findings suggest that NO within the NRM may play an important role in the regulation of skin blood flow.

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