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Cardiovascular effects of nitrergic system of the pedunculopontine tegmental nucleus in anesthetized rats

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ABSTRACT

Objective(s): Nitric oxide (NO) is an important neurotransmitter in central nervous system involved in central cardiovascular regulation. The presence of NO in the pedunculopontine tegmental (PPT) nucleus has been shown, but its cardiovascular effect has not been determined. In the present study, the cardiovascular effect of NO in the PPT nucleus was evaluated.

Materials and Methods: After induction of anesthesia, a polyethylene catheter (PE-50) filled with heparinized saline inserted into the femoral artery, and the blood pressure (BP) and heart rate (HR) were continuously recorded. Animals were then placed in a stereotaxic apparatus and maximum changes of mean arterial pressure (ΔMAP) and heart rate (ΔHR) after microinjection of two doses of N^G-nitro-L-arginine methyl ester (L-NAME, 30 and 90 nmol), L-arginine (L-Arg 10 and 50 nmol) and sodium nitroprusside (SNP, 9 and 27 nmol) into the PPT were provided and compared with control group (One-way ANOVA).

Results: Both doses of L-NAME significantly increased ΔMAP compared to control ($P<0.05$ and $P<0.01$, respectively). ΔHR only in higher dose (90 nmol) significantly increased compared to control ($P<0.05$). Two doses of L-Arg (10 and 50 nmol/150 nl) had no significant effect on ΔMAP or ΔHR. Higher dose of SNP (27 nmol) significantly decreased ΔMAP ($P<0.05$) and its both doses significantly decreased ΔHR compared to control ($P<0.05$ and $P<0.001$, respectively). Effect of higher dose on ΔHR was significantly higher than the lower dose ($P<0.05$).

Conclusion: Our results show an inhibitory effect of the nitrergic system of the PPT on central cardiovascular system.

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Introduction

The pedunculopontine tegmental (PPT) nucleus, a mesencephalic nucleus, participates in several functions including motor control, rapid eye movement sleep (REM), orientation, attention and autonomic regulation (1, 2). The role of PPT in the regulation of cardiovascular events has also been shown in previous studies (2, 3). It has been shown that microinjection of glutamate into the PPT nucleus evoked cardiovascular responses (2). In addition, previous studies have shown that the PPT is connected with regions, such as rostral ventrolateral medulla (RVLM), the hypothalamus nuclei, periaqueductal gray matter (PAG), nucleus tractus solitarius (NTS), cuneiform nucleus (CnF) and raphe nuclei that are involved in the cardiovascular regulation (3-6).

Nitric oxide (NO) is a well-known regulatory molecule with several physiological and pathological functions (7, 8). Hypotensive effect of NO on cardiovascular system has been previously shown (9). In addition, inhibition of NO synthesis by oral administration of N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase (NOS), caused sustain increase of blood pressure (BP) and is known as a model for induction of hypertention (10, 11).

Central cardiovascular effect of the nitrergic system has also been identified in several studies (9, 12-15) .The intracerebroventricular (ICV) injection of L-Arg (precursor of NO) increased NO synthesis within the CNS and reduced abdominal sympathetic nerve discharge in rats (8). The presence of NO in certain nuclei involved in cardiovascular regula-tion (such as RVLM, NTS, and paraventricular nucleus (PVN) has also been shown (15, 16). Unlike NOS inhibitors such as L-NAME, microinjection of L-arginine (L-Arg, a precursor of NO) into the RVLM decreased BP (17). There is also evidence that NO

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has a modulatory effect on the sympathetic nervous system (18). Presence of NO in the PPT nucleus has been previously shown (19). It is also reported a cholinergic projection of the PPT RVLM, an important area in cardiovascular regulation (6). Neurons of these cholinergic projections in addition to synthesis of acetylcholine (Ach) are also capable to synthesize NO (19).

Due to the presence of NO in the PPT and its co-localization with Ach in the PPT cholinergic projections to central cardiovascular areas, it is suggested that the nitrergic system of the PPT is involved in central cardiovascular regulation. Therefore, this study was performed to evaluate the effects of this system on cardiovascular responses in the PPT nucleus.

Materials and Methods

Animals and drugs

In present study, sixty male Wistar rats (200–250 g) were provided from Mashhad University of the Medical Sciences animal center. The animals were housed at room temperature (22±2 °C), on a 12 hr light/dark cycle. Food and water were available *ad libitum* properly.

The drug and reagents including urethane, L-NAME (an inhibitor of NOS), L-Arg (a precursor of NO) and sodium nitroprusside (SNP, a donor of NO) were provided from Sigma Chemical Company, USA. All drugs dissolved in saline.

Surgery and microinjection of the drugs

The rats were anesthetized intraperitoneally with urethane (1.4 g/kg), and supplementary doses (0.7 g/kg). A polyethylene catheter (PE-50) filled with heparinized saline was inserted into the left femoral artery. The mean arterial pressure (MAP) and heart rate (HR) were continuously recorded by a power lab system (ID instrument, Australia). After cannulation, the animals were placed in a stereotaxic apparatus (Stoelting, USA). The scalp was incised and the skull was leveled between lambda and bregma, and a small hole drilled in the skull. The stereotaxic coordinates of the PPT were -7.6 to -8.5 mm caudal to bregma, -1.8 to -2.2 mm lateral to the midline suture and -6.8 to -7.8 mm ventral from the bregma according to the atlas of Paxinos and Watson (20). Drug microinjection into the PPT nucleus was performed by a single barreled micropipette with an internal diameter ranging 35–45 µm. The micropipette connected through a PE-10 tube to an injection syringe and was carefully introduced into the PPT and injection was performed. Injections volume in all groups was 150 nl that injected in 30 sec (21, 22). The protocol of study was approved by the Bioethics committee of Mashhad University of Medical Sciences (ID, 922758).

The following groups were used in this study:

1- The control group: Microinjection of vehicle (normal saline) into the PPT
2,3 L-NAME groups: Microinjection of L-NAME (30 and 90 nmol) (23-25)
4, 5- L-Arg groups: Microinjection of L-Arg (10 and50 nmol) (25, 26)
6,7- SNP groups: Microinjection of sodium nitroprusside (9 and 27 nmol)(27)

Data analysis

The data of BP and HR values were expressed as mean±SEM. The maximal changes of ΔMAP and ΔHR in each group were provided and compared with the control group using the one-way ANOVA followed by Tukey's *post hoc* test. The changes of ΔMAP and ΔHR between two doses were also compared by independent-samples t test. $P<0.05$ was used to indicate statistical significance.

Histological procedure

At the end of each experiment, the injection sites were marked by up and down movement of micropipette to construct an obvious track (28). The brains were perfused transcardially with 100 ml of 0.9% saline, followed by 100 ml of 10% formalin. After that, the animals were sacrificed by high dose of urethane. The brains were removed and stored in 10% formalin for at least 24 hr at 4 ^{o}C. Serial sections (60 µm) were prepared and the locations of the injection sites (29) were verified according to a rat brain atlas (20) under the light microscope (29).

Results

Microinjection of the saline (100-150 nl, n = 10) into the PPT showed that there were no significant differences in changes of MAP (Before: 93.4 ± 5.30 mmHg, after: 95. 8 ± 6.3 mmHg) and HR (Before: 312.4 ± 9.5 beats/min, after: 318.7 ± 10.5 beats/min) before and after injection.

To determine the role of NO in the cardiovascular system, in first experiment, two doses of L-NAME, an inhibitor of NOS, (30 and 90 nmol/150 nl) were microinjected into the PPT. Tracing of cardiovascular responses after injection of L-NAME has been shown in Figure 1. As shown, both doses of L-NAME increased BP and HR. Maximal ΔMAP in both doses were significantly higher compared to control group (dose 30: $P<0.05$; n= 9 and dose 90: $P<0.01$, n= 10; one way ANOVA, Figure 2 a). Comparing the effect of two doses of L-NAME indicated that the effect of high dose on ΔMAP is significantly higher than the low dose ($P<0.05$, independent-samples t test). Both doses of L-NAME increased ΔHR compared to control group, but the only effect of higher dose on ΔHR was significant compared to the control group (Dose 30: $P>0.05$; n= 9 and Dose 90: $P<0.01$, n= 10, Figure 2b). The ΔHR in higher dose was also significant compared to the lower dose (dose ($P<0.05$; independent-samples t test).

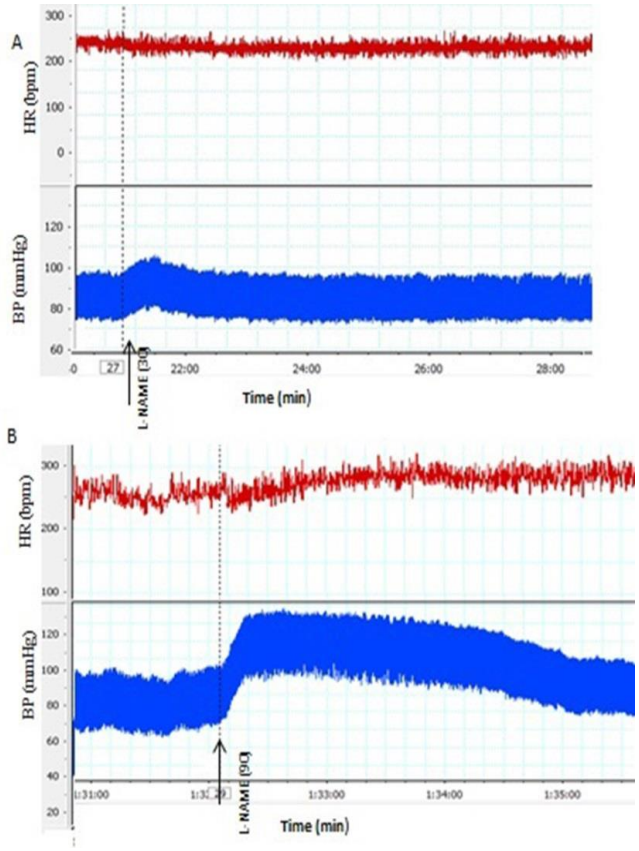

Figure 1. Samples of changes of blood pressure (BP) and heart rate (HR) to microinjection of lower (A) and higher (B) doses of N^G-nitro-L-arginine methyl ester (L-NAME) into the pedunculo-pontine tegmental (PPT) nucleus
The vertical lines indicate the injection time

In second experiment two doses of L- Arg (10 and 50 nmol/150 nl), were microinjected into the PPT. Figure 3 shows a tracing of cardiovascular responses after injection of L-Arg. Maximal ΔMAP of two doses were not significant compared to the control group (P>0.05; one way ANOVA; n=8, Figure 4a). The HR changes of both doses of L- Arg also decreased ΔHR. However, these effects were not significant compared to the control group (P>0.05; one way ANOVA, n=8, Figure 4b).

In third experiment, two doses of SNP, a donor of NO, microinjected into the PPT nucleus. Figure 5 shows a sample of cardiovascular responses after injection of SNP. Maximal changes of two doses are shown in Figure 6 .As shown, both doses of SNP decreased maximal ΔMAP compared to control group, but only effect of higher dose was significant (P<0.05; one way ANOVA; n=8, Figure 6a). Both doses of SNP significantly decreased ΔHR compare to control group (dose 9: P<0.05; n=7 and Dose 27: P<0.001, n= 8; Figure 6 b). However, effect of higher dose on ΔHR was also significant compare to the lower dose (P<0.01; independent-samples t test).

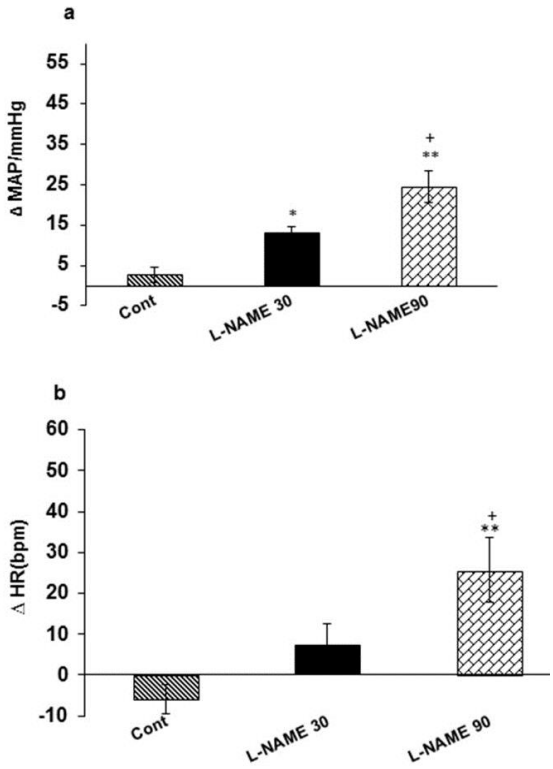

Figure 2. Maximal changes of mean arterial pressure (ΔMAP) and heart rate (ΔHR) in response to microinjection of two doses of N^G-nitro-L-arginine methyl ester (L-NAME) (30 and 90 nmol) into the pedunculopontine tegmental (PPT) nucleus n=11 a; MAP, b; HR *; P<0.05, **; P<0.01 compare to control group (One-way ANOVA followed by Tukey's *post hoc* test) +; P<0.05 dose 30 compare to dose 90 (independent-samples t test)

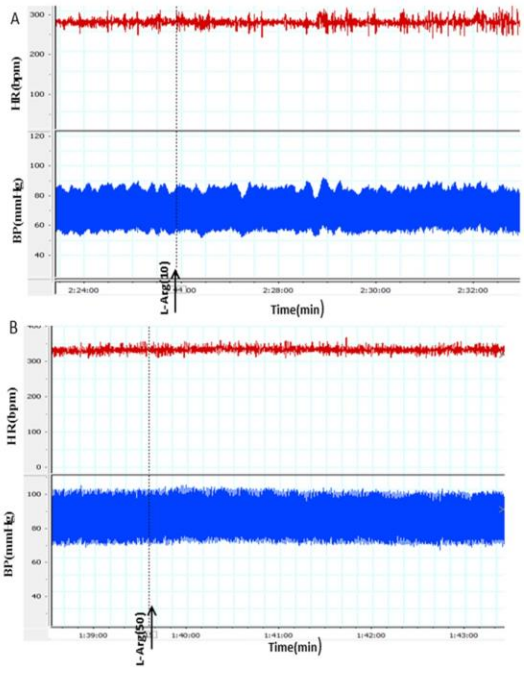

Figure 3. Samples of changes of blood pressure and heart rate to microinjection of lower (A) and higher (B) doses of L-arginine (L-Arg) into the pedunculopontine tegmental (PPT) nucleus.
The vertical lines indicate the injection time

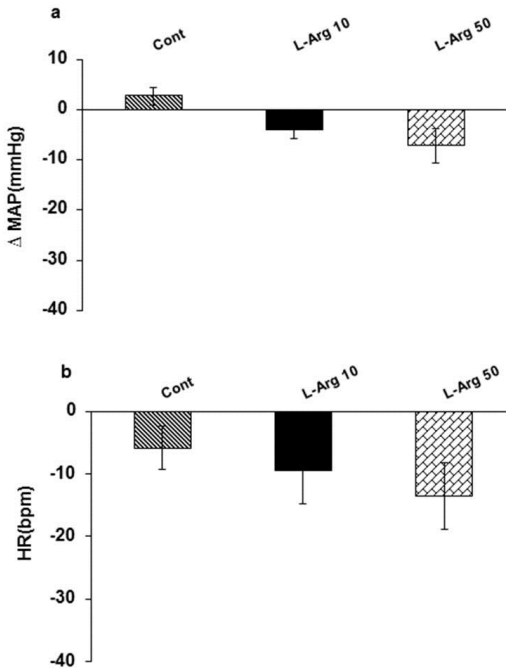

Figure 4. Maximal changes of mean arterial pressure (ΔMAP) and heart rate (ΔHR) in response to microinjection of two doses of L-arginine (L-Arg; 10 and 50 nmol) into the pedunculopontine tegmental (PPT) nucleus

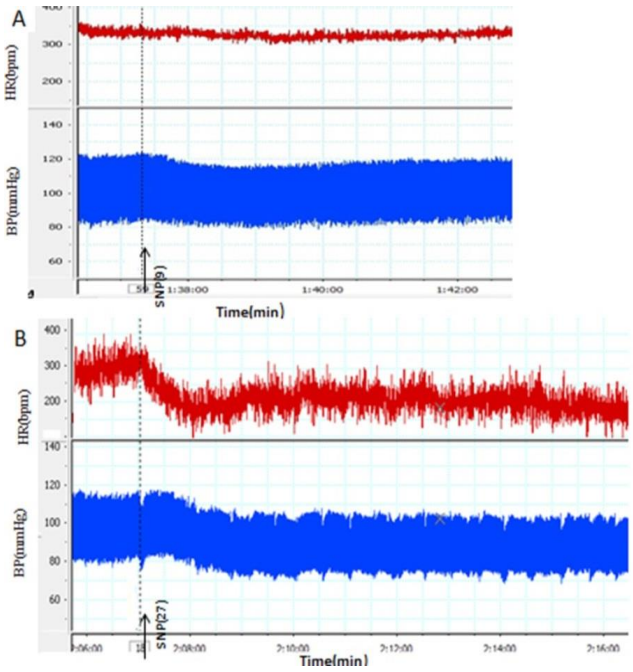

Figure 5. Samples of changes of blood pressure (BP) and heart rate (HR) to microinjection of lower (A) and higher (B) doses of sodium nitroprusside (SNP) into the pedunculopontine tegmental (PPT) nucleus; The vertical lines indicate the injection time

Discussion

The cardiovascular effect of NO has been well-known in several nuclei such as RVLM and NTS (30-34). However, this effect of NO in the PPT nucleus is not determined. The result of present study showed that nitrergic system of the PPT nucleus has an inhibitory effect on the central cardiovascular system, so microinjection of L-NAME, a NOS inhibitor, into the PPT increased MAP and HR, while NPS decreased MAP and HR, and L- Arg has no significant effect on these cardiovascular values. The cardiovascular effect of NO in the PPT nucleus is unknown. However, it is speculated that these effects are complicated and may be mediated by several mechanisms. It has been shown that NO by increasing cyclic guanosine monophosphate (cGMP) modulate vasomotor neurons activity (15, 16, 35). The NO has also an inhibitory effect on the sympathetic system (18). Because the PPT has projection to RVLM; an important sympatho-excitatory area in the medulla (36), it is conceivable that inhibitory effect of nitrergic system of the PPT is mediated via effect on vasomotor neurons of the RVLM. The cardiovascular function of the sympathetic system is regulated by pre-sympathetic motor neurons located in the several brain areas including brain stem (36). These brain areas have a vigorous effect on regulation of cardiovascular responses (37). In consistent with our opinion, the result of a previous study has shown that microinjection of L-NAME into the RVLM increased

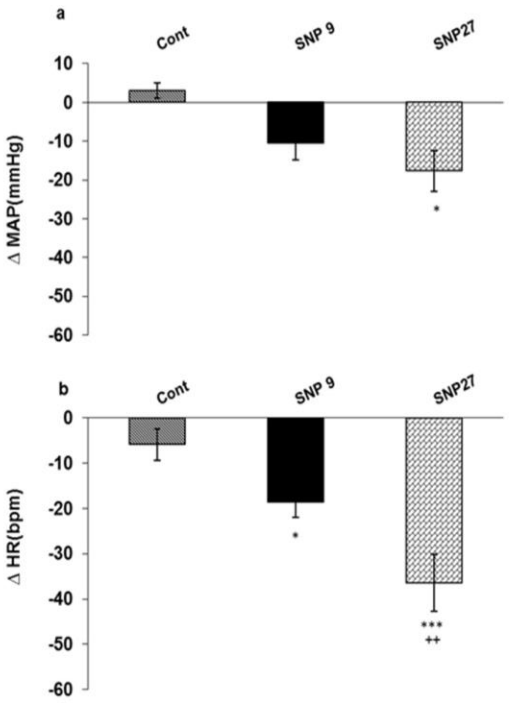

Figure 6. Maximal changes of mean arterial pressure (ΔMAP) and heart rate (ΔHR) in response to microinjection of two doses of sodium nitroprusside (SNP, 9 and 27 nmol) into the pedunculopontine tegmental (PPT) nucleus
n =11 A; MAP, B; HR ;* $P<0.05$, **; $P<0.001$ Compare to control group (One-way ANOVA followed by Tukey's *post hoc* test)
++; $P<0.01$ Dose 9 compare to dose 27 (independent-samples t test)

blood pressure and sympathetic nerve activity, and microinjection of L-Arg and NPS decreased these variables (17). In our study, microinjection of SNP decreased cardiovascular responses, while L-NAME increased these responses. Because SNP is a donor of NO, it is suggested that presence of NO in the PPT has inhibitory effect on vasomotor of the sympathetic system. The L-NAME decreased inhibitory effect of NO on vasomotor of sympathetic system and increased blood pressure by blocking the production of NO. Because microinjection of the L-Arg into the PPT nucleus did not change cardiovascular parameters, we suggested that L-Arg of the PPT in basal condition cannot be converted to NO by NOS. Previously, a cholinergic projection from the PPT to RVLM has been reported (3). These cholinergic neurons of the PPT, beside synthesis of Ach, could also be involved in synthesis of the NO (38). Due to co-localization of NO and Ach, we suggested that NO released from terminals of this projection PPT cholinergic neurons diffuses on the vasomotor neurons of sympathetic system in RVLM and decreases cardiovascular responses by inhibitory effect on these neurons. A non- cholinergic projection from the PTT to several areas has also been reported (39). So, another possibility is that cardiovascular effect of NO is mediated by this projection.

The presence of several neurotransmitters involved in cardiovascular regulation such as glutamate, gamma-aminobutyric acid (GABA) and Ach has been reported in the PPT nucleus (40, 41). Therefore, it is proposed that modulation of the cardiovascular responses of NO in the PPT is mediated by interaction with these neurotransmitters. One abundant neurotransmitter in the PPT nucleus is Ach. Interaction of Ach, NO and sympathetic nervous system in controlling the cardiovascular responses has been demonstrated (42). Therefore, interaction of NO with Ach is also suggested in cardiovascular effect of the PPT nucleus. There is also evidence that inhibitory effect of NO on cardiovascular responses is partly mediated by GABAergic system. For example, Zhang and Patel in 1998 reported that inhibitory effect of NO in the PVN nucleus is mediated by the GABAergic system (43). Because GABA neurotransmitter is also present in the PPT, we suggested that the effect of NO in the PPT nucleus may be partly mediated by interaction with GABAergic system. Interaction of NO with glutamate in the central cardiovascular regulation system has also been shown in some previous studies (44, 45). Presence of glutamate in the PPT has been reported, and it has been shown that microinjection of glutamate into the PPT can induce pressor response (2). In addition, it is reported that NO donors decreased depolarization effect of NMDA receptor of glutamate in the PPT neurons (19). Based on these evidences, interaction of NO with glutamate in controlling the cardio-vascular response of the PPT can also be speculated. Effect of NO on central regulation of cardiovascular responses is different depending on conditions. For example, nitrergic system of RVLM in anesthetized rats shows inhibitory effect on the cardiovascular system (17). But, a pressor and sympathoexcitatory effect has been shown in conscious rat (46). However, our study was performed in anesthetized rats; therefore, it is reasonable that the effect of NO in the PPT in anesthetized and conscious rats is different. However, future studies needed to clarify this opinion. Previous studies showed that the PPT project to several areas involved in cardiovascular regulation such as NTS, PAG, raphe nuclei, PVN and lateral hypothalamus (5). It is possible that cardiovascular effect nitrergic system of the PPT is indirect and mediated by these areas. The PPT participates in several functions including control of movement, respiratory regulation and behavioral functions (3). There are also evidences that local neural networks are present in the PPT nucleus that each one regulates a special function (2, 47). Based on these observations, we suggest that a local network related to cardiovascular regulation is also formed in the PPT nucleus and NO has modulatory effect on this local network.

Involvement of the PPT nucleus in both waking and sleep states has also been reported (38). The results of an electrophysiological study have shown that firing rate of one group of the PPT neurons in active wake is higher than sleep (48). Because the PPT involves in waking–sleep cycle and cardiovascular regulation (3, 38), it is speculated that this groups of neurons are nitrergic and beside projection to waking- sleep areas have a projection to central cardiovascular areas and participate in the central control of cardiovascular responses in waking –sleep cycle. However, further works needed to clarify this opinion.

Conclusion

In summary, the present study for first time provides evidence that nitrergic system of the PPT nucleus has an inhibitory effect on basal cardiovascular responses.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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