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Original Article

Role of the Nitrergic System of the Cuneiform Nucleus in Cardiovascular Responses in Urethane-Anesthetized Male Rats

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Abstract

Background: The presence of nitric oxide (NO) in the cuneiform nucleus (CnF) has been previously shown. In this study, NG-nitro-L-arginine methyl ester (L-NAME) (an inhibitor of NO synthase), L-arginine (L-Arg) (a precursor of NO), and sodium nitroprusside (SNP) (a donor of NO) were microinjected into the CnF and cardiovascular responses were investigated.

Methods: Seventy male rats were divided into 7 groups (n=10 each): 1) saline, 2 and 3) L-NAME (30 and 90 nmol), 4 and 5) L-Arg (20 and 60 nmol), and 6 and 7) SNP (9 and 27 nmol). After anesthesia, the femoral artery was cannulated and cardiovascular parameters were recorded using a PowerLab system. Time course changes in mean arterial pressure (ΔMAP) and heart rate (ΔHR) were calculated and compared with those in the control group (repeated measures ANOVA). Maximum ΔMAP and ΔHR were also compared with those in the control group (independent sample *t* test).

Results: ΔMAP with both doses of L-NAME (30: P=0.026 and 90: P=0.007) and ΔHR with the higher dose (P=0.034) were significantly higher than those in the control group. Maximal ΔMAP with both doses (P<0.01 and P<0.001, n=10) and maximal ΔHR with the higher dose (P<0.01) were significantly higher than those in the control group. Changes in L-Arg with both doses were not significantly higher than those in the control group (P=0.26, n=8). ΔMAP and ΔHR of SNP only with the higher dose were significantly lower than those in the control group (P=0.006 and P=0.035), and maximal responses with the higher dose were lower than those in the control group (ΔMAP: P<0.01 and ΔHR: P<0.05, n=7).

Conclusion: Our results showed that the nitrergic system of the CnF had an inhibitory effect on central cardiovascular regulation.

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Keywords ● Cuneiform nucleus ● Nitroprusside ● Nitric oxide ● Blood pressure ● NG-nitro-L-arginine methyl ester

What's Known

- Nitric oxide (NO) in certain nuclei is involved in cardiovascular regulation. Cuneiform nucleus (CnF) is a mesencephalic nucleus involved in cardiovascular regulation.
- Presence of NO in CnF also has been reported.

What's New

- Nitrergic neurons in CnF are involved in central cardiovascular regulation.
- These neurons induce an inhibitory effect on cardiovascular activity.

Introduction

Nitric oxide (NO) is a gaseous molecule with important physiological and pathological functions.[1-4] NO is synthesized from L-arginine (L-Arg) by 3 isoforms of nitric oxide synthase (NOS): endothelial NOS, neuronal NOS, and inducible NOS.[2,5]

NO has a well-known role in the regulation of the cardiovascular system via peripheral and central effects.[4,6] With respect to the peripheral effects, NO is synthesized mostly in the endothelium and by increasing cyclic guanosine monophosphate produces vascular relaxation.[7] NO is also needed for the regulation of basal vascular tone.[8] Previous studies have indicated that the inhibition of NO production in the vessels leads to hypertension.[8,9] For example, the systemic administration of NG-nitro-L-arginine methyl ester (L-NAME), which is an inhibitor of NOS, sustains increased blood pressure.[10]

The central cardiovascular effects of NO have also been identified in several studies.[11,12] The intracerebroventricular injection of L-NAME in anesthetized rats was reported to have increased both heart rate and arterial blood pressure.[13] The injection of L-arginine (L-Arg) (a precursor of NO) was also reported to have increased NO synthesis within the central nervous system and reduced abdominal sympathetic nerve discharge in rats.[13] Moreover, there is evidence that NO has a modulatory effect on the sympathetic nervous system.[5] The presence of NO in certain nuclei involved in cardiovascular regulation such as the rostral ventrolateral medulla (RVLM),[14] nucleus tractus solitarius (NTS),[15] and paraventricular nucleus[16] has also been previously shown.

The cuneiform nucleus (CnF) is a sympathoexcitatory nucleus located in the mesencephalic area and involved in cardiovascular regulation.[17-19] Previous research has indicated a relation between the CnF and the central areas involved in cardiovascular modulation including the RVLM, NTS, periaqueductal gray matter, and parabrachial/Kölliker-Fuse complex.[20] The presence of several neurotransmitters such as glutamate and acetylcholine has been reported in the CnF. Our previous study demonstrated that the stimulation of the CnF with glutamate elicited a pressor effect with bradycardia or tachycardia effects.[17] In addition, a microinjection of acetylcholine caused a depressor effect with no significant effects on heart rate.[21] NO is another important neurotransmitter of the CnF,[22] but its role in cardiovascular regulation has yet to be fully elucidated. Because the CnF is a sympathoexcitatory region and NO has an inhibitory effect on the sympathetic nervous system,[23] we hypothesized that NO in the CnF is involved in cardiovascular regulation via an effect on the sympathetic system or interaction with the neurotransmitters. Accordingly, in the present study, we primarily investigated the effects of the nitrergic system of the CnF on the central cardiovascular system.

Materials and Methods

Animals and Drugs

Experiments were performed on 70 male Wistar rats (220–270 g). The animals were provided by the Animal House of Mashhad University of Medical Sciences. The animals were housed in standard cages (4–5 per cage) at room temperature (22±2°C), in a 12-hour light/dark cycle. Food and water were available *ad libitum*. Animal surgery and all the related procedures were approved by Mashhad Medical University's Committee on Animal Research.

The drugs, consisting of urethane, L-NAME, L-Arg, and sodium nitroprusside (SNP), were provided by Sigma Chemical Company (USA). All the drugs were dissolved in saline.

Surgery and Microinjection of the Drugs

After anesthesia with intraperitoneal 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 femoral artery. The mean arterial pressure and heart rate were continuously recorded using a PowerLab system (ID Instrument, Australia). The animals' temperature was kept at 37°C with a heating lamp.

A small hole was drilled in the skull over the CnF according to the stereotaxic coordinates of the Rat Brain in Stereotaxic Coordinates by Paxinos and Watson (7.6–8.5 mm caudal to the bregma, 1.7–2.2 mm lateral to the midline suture, and 5.5–6.2 mm ventral from the bregma).[24]

The drugs (100–150 nL) were microinjected into the CnF using a single-barreled micropipette with an internal diameter of 35 to 45 µm. The injection was done with a manual injector (Stoelting, USA). An ocular micrometer (Waltex, China) was also used to directly measure the volume of drug injection.

Experimental Groups

The study groups were as follows (n=10 per group):

1) Control group: injection of saline into the CnF
2, 3) L-NAME groups: injection of L-NAME (30 or 90 nmol) into the CnF separately
4, 5) L-Arg groups: injection of 2 doses of L-Arg (20 or 60 nmol) into the CnF separately
6, 7) SNP groups: injection of 2 doses of SNP (9 or 27 nmol) into the CnF separately

Data Analysis

The data on blood pressure and heart rate values are expressed as means±SEMs. Changes in mean arterial pressure (ΔMAP) and changes

in heart rate (ΔHR) after the microinjections of the reagent and drugs at different intervals (time courses) within each group and between each group were calculated and compared with those in the control group (repeated measures ANOVA). Additionally, maximum ΔMAP and ΔHR were provided and compared with those in the control group (independent sample *t* test). A P<0.05 was used to indicate statistical significance.

Histological Procedure

At the end of each experiment, the injection sites were marked and the animals were sacrificed after anesthesia with a high dose of urethane. The brains were perfused transcardially with 100 mL of 0.9% saline, followed by 100 mL of 10% formalin. After 30 minutes, the brains were carefully removed and placed in 10% formalin for 24 hours. Serial sections (60 µm) were prepared, and the locations of the injection sites were verified according to a rat brain atlas under a light microscope.[25]

Results

Effects of the Microinjection of Saline into the Cuneiform Nucleus on Blood Pressure and Heart Rate

The microinjection of saline (100–150 nL, n=10) into the CnF did not affect ΔMAP (before: 72.1±4.59 mm Hg, after: 76.4±3.3 mm Hg) or ΔHR (before: 324.8±6.4 beats/min, after: 336±6.2 beats/min).

Effects of the Microinjection of NG-nitro-L-Arginine Methyl Ester into the Cuneiform Nucleus on Blood Pressure and Heart Rate

In this experiment, the cardiovascular effects of L-NAME (an NOS inhibitor) were examined. Two doses of L-NAME (30 and 90 nmol) were separately microinjected into the CnF. A sample tracing of cardiovascular responses after the injection of L-NAME is shown in figure 1. Additionally, ΔMAP and ΔHR after the microinjection of 2 doses of L-NAME are depicted in figure 2. As is shown, both doses of L-NAME significantly increased ΔMAP compared to that in the control group (P=0.026 and P=0.007, respectively; repeated measures ANOVA). The maximal changes with both doses were also significantly higher than those in the control group (maximal ΔMAP dose 30: 14.24±3.9 mm Hg vs. -4.5±1.0 mm Hg, P<0.01; n=9; maximal ΔMAP dose 90: 26.03±4.45 mm Hg vs. -4.5±1.0 mm Hg, P<0.001; n=10, independent *t* test). Both doses of L-NAME (30 and 90 nmol) decreased ΔHR, but only the effect with the higher dose (90 nmol) was significantly higher than that in the control group (P=0.034, repeated measures ANOVA). The maximal change in ΔHR with the higher dose was significantly lower than that in the control group (maximal ΔHR 80: -34.63±7.39 beats/min vs. -9.6±2.7 beats/min, P<0.01; independent sample *t* test; n=10).

Effects of the Microinjection of L-Arginine into the Cuneiform Nucleus on Blood Pressure and Heart Rate

To determine whether the injection of L-Arg (a precursor of NO) in the CnF had an effect on the cardiovascular system, we microinjected 2 doses of L-Arg (20 and 60 nmol, 100–150 nL) into the CnF. Figures 3 and 4 illustrate a sample tracing and ΔMAP and ΔHR after the microinjection of L-Arg. As is demonstrated, the changes in ΔMAP with the 2 doses (20 and 60 nmol) were not significantly higher than those in the control group (P=0.26, n=8, repeated measures ANOVA). The maximal changes with

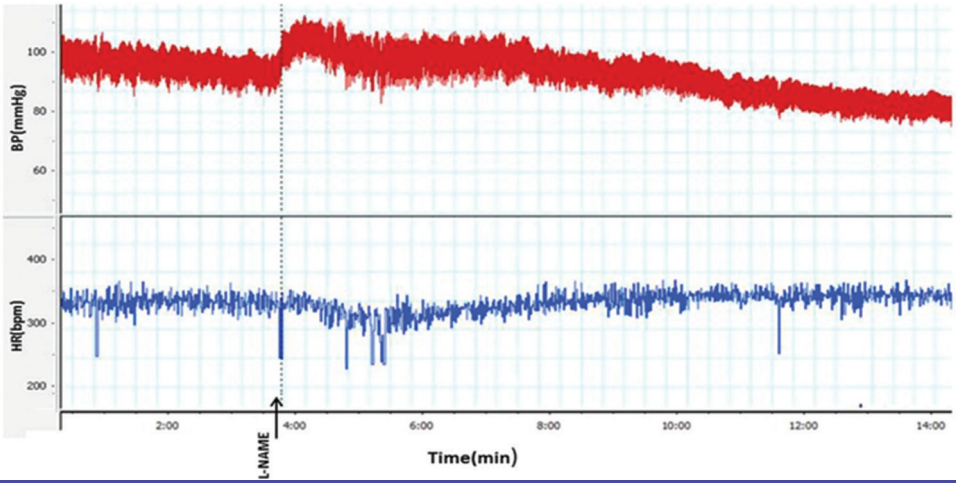

Figure 1: A sample of blood pressure and heart rate, indicating cardiovascular responses to the microinjection of L-NAME into the CnF.

both doses also were not significantly higher than those in the control group (dose 20: -9.2±1.6 mm Hg vs. -4.5±1.0 mm Hg; dose 60: -11.53±3.3 mm Hg vs. -4.5±1.0 mm Hg; n=8). The changes in ΔHR with both doses of L-Arg (20 and 60 nmol) also were not significantly higher than those in the control group. The maximal changes in ΔHR with both doses also were not significant higher than those in the control group (dose 60: -20.14±5.4 beats/min vs. -9.6±4.2 beats/min, P>0.05, n=8, independent sample *t* test).

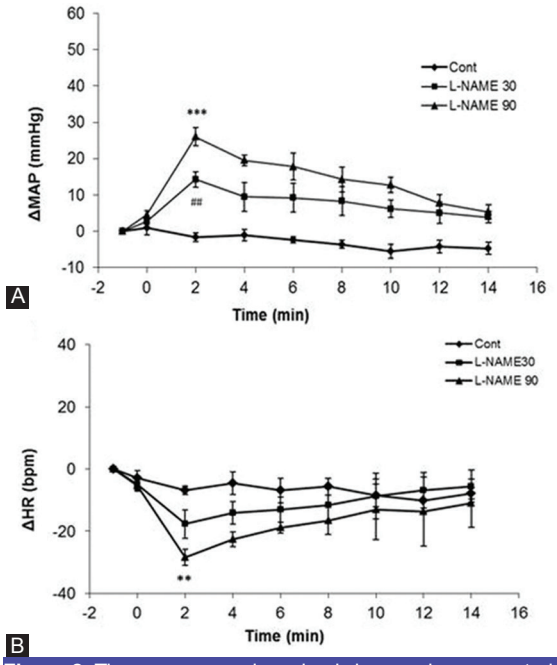

Figure 2: Time courses and maximal changes in mean arterial pressure (ΔMAP) (A) and heart rate (ΔHR) (B) in response to the microinjection of 2 doses of L-NAME (30 and 90 nmol) into the CnF. Changes in ΔMAP with both doses (P=0.26 and P=0.007) and changes in ΔHR with the high dose (P=0.034) were significantly higher than those of the control group (repeated measures ANOVA). Maximal ΔMAP and maximal ΔHR were significantly higher than those in the control group. **; P<0.01, ***; P<0.01; maximal changes of dose 90 vs the control ##; P<0.01; maximal changes of dose 30 vs. the control.

Effects of the Microinjection of Sodium Nitroprusside into the Cuneiform Nucleus on Blood Pressure and Heart Rate

In this experiment, NPS (a donor of NO) was microinjected into the CnF. The administration of the 2 doses of SNP (9 and 27 nmol) caused a decrease in ΔMAP and ΔHR. A sample tracing and ΔMAP and ΔHR after the microinjection of SNP in the CnF are shown in figures 5 and 6. Both doses of SNP decreased ΔMAP and ΔHR; however, only the changes with the higher dose (27 nmol) were significantly lower than those in the control group (ΔMAP: P=0.006 and ΔHR: P=0.035, repeated measures ANOVA, n =7). Maximal ΔMAP and maximal ΔHR only with the higher dose (27 nmol) were significantly lower than those in the control group (maximal ΔMAP dose 27: -19.31±4.94 mm Hg vs. -4.5±1.0 mm Hg, P<0.01; and maximal ΔHR dose 27: -18.7±5.5 beats/min vs. -9.6±4.2 beats/min, P<0.05; independent sample *t* test, n=7).

Discussion

In this experiment, the effects of NO in the CnF on the cardiovascular system were evaluated. We used L-NANE and L-Arg to evaluate NOS activity and NO production and SNP for the direct release of NO in the CnF. Our results showed that a microinjection of L-NAME increased ΔMAP and decreased ΔHR. L-Arg had no significant effects on ΔMAP and ΔHR, and a higher dose of

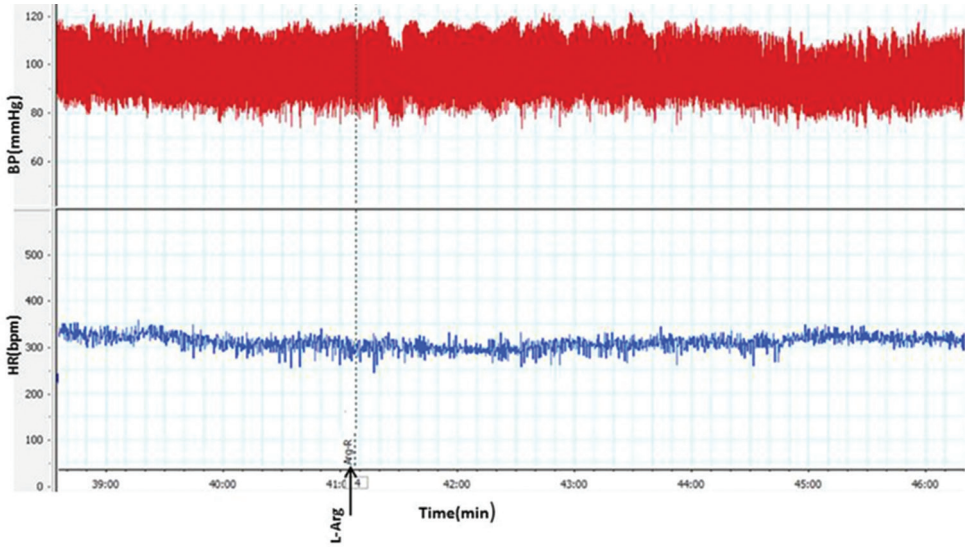

Figure 3: A sample of blood pressure and heart rate, indicating cardiovascular responses to the microinjection of L-Arg into the CnF.

SNP decreased ΔMAP and ΔHR.

To the best of our knowledge, the result of the present study showed for the first time the effects of the nitrergic system of the CnF on the central cardiovascular regulation. The central effects of NO on controlling the cardiovascular system have been shown in previous studies.[26-29] The mechanism of NO in the central regulation of the cardiovascular system is complicated and mediated by several methods. In several studies, NO is known as a neurotransmitter in the brain that affects the firing activity of vasomotor neurons or has an inhibitory effect on the sympathetic system.[5] Furthermore, NO has several interactions with several neurotransmitters such as glutamate, gamma-aminobutyric acid (GABA), and acetylcholine in the brain areas.[5]

Pose et al.[30] (2000) reported a distinct group of neurons containing NOS–NADPH-diaphorase (NADPH-d) in the CnF. The mechanism of the effects of NO in the CnF is unknown; it has, however, been postulated that it is mediated by several ways.

The CnF is a sympathoexcitatory nucleus and its effect is mostly mediated by direct and/or indirect projection to the RVLM.[17] The RVLM is the main source of premotor neurons that project to the sympathetic preganglionic neurons in the spinal cord and has profound effects on cardiovascular regulation.[31] It has been reported that the injection of L-Arg into the RVLM elicited depressor and bradycardic effects, while L-NAME increased blood pressure and ΔHR.[32,33] Furthermore, these effects of NO in the RVLM area were mediated by the inhibition of the sympathetic system. Similar to the RVLM area, the inhibitory effects of the NO system on the sympathetic system in the CnF and the following inhibitory effects on the cardiovascular system might be suggested. In addition, the presence of important neurotransmitters involved in cardiovascular regulation such as glutamate, GABA, and acetylcholine has been shown by immunocytochemical and pharmacological studies in the CnF.[17,34,35] Because the interactions between NO and the mentioned neurotransmitters have been previously reported, it is possible that NO interacts with these neurotransmitters in the CnF. In our previous study, we showed that a microinjection of glutamate into the CnF was able to produce 2 short and long pressor effects with bradycardic and tachycardic responses, respectively.[17] Ishide et al.[36] indicated an interaction between glutamate and NO in the

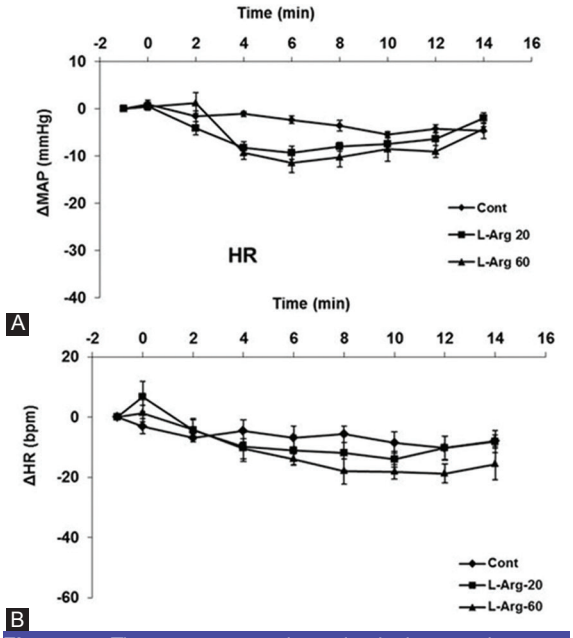

Figure 4: Time courses and maximal changes in mean arterial pressure (ΔMAP) (A) and changes in heart rate (ΔHR) (B) in response to the microinjection of 2 doses of L-Arg (20 and 60 nmol) into the CnF. Changes in ΔMAP and ΔHR with both doses were not significantly higher than those in the control group (repeated measures ANOVA). Maximal ΔMAP and maximal ΔHR were not significantly higher than those in the control group (independent sample *t* test, n=8).

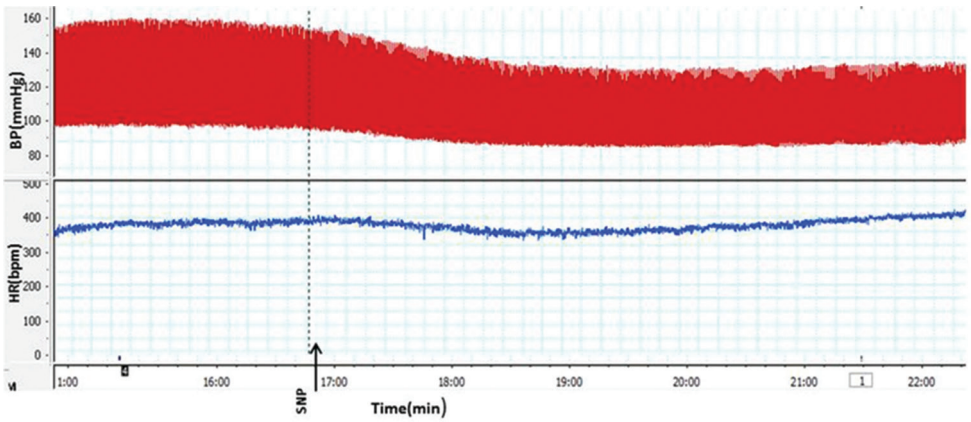

Figure 5: A sample of blood pressure and heart rate, indicating the cardiovascular responses to the microinjection of SNP into the CnF.

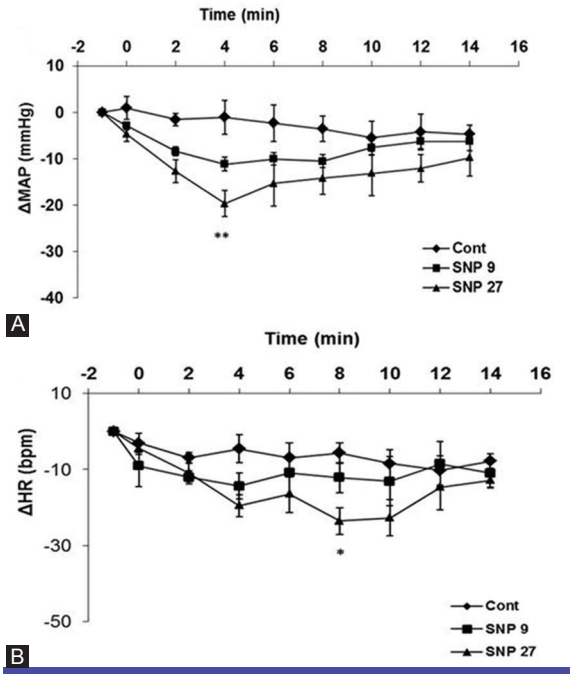

Figure 6: Time courses and maximal changes in mean arterial pressure (ΔMAP) (A) and changes in heart rate (ΔHR) (B) in response to the microinjection of 2 doses of SNP (9 and 27 nmol) into the CnF. Changes in ΔMAP (P=0.006) and ΔHR(P=0.035) with the higher dose were significantly lower than those in the control group (repeated measures ANOVA). Maximal ΔMAP and maximal ΔHR were significantly lower than those in the control group (independent sample *t* test, n=7). *; P<0.05, **; P<0.01 maximal changes of dose 27 vs. the control.

RVLM; it is, therefore, possible to suggest an interaction between glutamate and NO in the CnF.

The hypotensive effect of the cholinergic system in the CnF was also reported in our previous study.[21] A possible mechanism for the effect of NO is its interaction with acetylcholine. Satori et al.[37] (2005) reported an interaction between NO and the cholinergic system in cardiovascular regulation.[37] Zhang et al.[16] (2003) also reported that there were NO–GABA interactions in the paraventricular nucleus neurons that project to the RVLM and the NTS. On the basis of this evidence, we suggest that NO exerts its cardiovascular effects through interaction with GABA and acetylcholine.

Previous studies have demonstrated that the Kölliker-Fuse complex is an important area in relaying the cardiovascular effects of the CnF to the RVLM. Because the Kölliker-Fuse complex contains NO,[38] it is possible that the nitrergic neurons of the CnF have a modulatory effect on the Kölliker-Fuse complex projection to the RVLM. The CnF also has a relation with the periaqueductal gray matter and the NTS.[20,34,39] All of these areas are involved in blood pressure; therefore, the effects of NO may be mediated by these areas.

Theoretically, the cardiovascular effects of SNP, or L-Arg, may be in contrast with the effects of L-NAME. However, our results showed that a microinjection of L-NAME into the CnF in contrast with SNP increased blood pressure but reduced HR. The mechanism(s) of this effect of NO is not defined. Nonetheless, based on previous research indicating that the regulation of blood pressure and HR in the CnF is separate,[17-19] it might be suggested that the effect of NO on HR, which was seen in the present study, is different from that of blood pressure. There is a relationship between the CnF and the nucleus of the amygdala and the dorsal motor nucleus, which are involved in HR regulation.[20,39] Bradycardia response may also be a result of evoked baroreflex function.

To test whether the precursor of NO in the CnF has an effect on cardiovascular responses, we microinjected L-Arg into the CnF. Our results showed that L-Arg had no significant effects on cardiovascular parameters. It is suggested that NOS probably in the CnF is low or there is an adequate amount of NO precursor (L-Arg) in the CnF. Consequently, a microinjection of L-Arg has no effects on cardiovascular responses.

The cardiovascular effects of NO in the CnF have yet to be clarified, and further investigations are needed to evaluate how NO produces its cardiovascular effects in this nucleus and to determine whether or not it interacts with other neurotransmitters.

Conclusion

Our findings demonstrated that nitrergic neurons in the CnF were involved in central cardiovascular regulation and that these neurons induced an inhibitory effect on cardiovascular activity.

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Conflict of Interest: None declared.

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