Research Paper: Cardiovascular Effect of Cuneiform Nucleus During Hemorrhagic Hypotension

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ABSTRACT

Introduction: The underlying mechanism responsible for the cardiovascular response to Hemorrhage (HEM) is still unknown; however, several brain areas, such as the Cuneiform nucleus (CnF) have shown to be involved. In this study, the cardiovascular effect of the CnF during HEM was evaluated.

Methods: The animals were divided into the following groups: 1. Vehicle; 2. HEM; 3. Cobalt chloride ($CoCl_2$); 4. $CoCl_2$+saline; and 5. $CoCl_2$+HEM. Catheterization of the left and right femoral artery was performed to record blood pressure and blood withdrawal, respectively. Saline and $CoCl_2$ were microinjected into the CnF nucleus, and then blood withdrawal was done for HEM induction. Cardiovascular regulation throughout the experiments was recorded and changes (Δ) in the Systolic Blood Pressure (SBP), Mean Arterial Pressure (MAP) and Heart Rate (HR) were calculated over time and compared with those treated with saline and HEM, using repeated-measures ANOVA.

Results: HEM significantly reduced ΔSBP and ΔMAP and augmented ΔHR than the vehicle group. $CoCl_2$ did not significantly affect basic ΔSBP, ΔMAP, and ΔHR compared with the vehicle group. However, injection of $CoCl_2$ into the CnF before HEM ($CoCl_2$+HEM group) significantly decreased ΔSBP, ΔMAP, and tachycardia, induced by HEM.

Conclusion: Our results indicated that blockade of the CnF by $CoCl_2$ significantly reduced the hypotension and tachycardia, induced by HEM indicating the involvement of CnF in cardiovascular regulation during HEM.

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Highlights

● Inactivation of the CnF by CoCl$_2$ did not significantly affect basal cardiovascular responses.

● Hemorrhage significantly lowered blood pressure and increased heart rate.

● Inactivation of the CnF significantly attenuated hypotension and tachycardia induced by hemorrhage.

Plain Language Summary

The Hemorrhage (HEM) is a life-threatening condition produced by a significant loss of blood, leading to hypotension, decreased tissue perfusion, cellular hypoxia and possibly death. Following HEM, several systems, including the Central Nervous System (CNS) are activated to improve this condition. For example, CNS restores hypotension during HEM and increases tissue perfusion. Numerous brain areas, such as rostral ventrolateral medulla, nucleus tractus solitarius, paraventricular nucleus are involved in the regulation of cardiovascular responses during HEM. Also, other areas, like Cuneiform Nucleus (CnF) are involved in cardiovascular regulation; however, their cardiovascular effects during HEM are unclear. In this study, we evaluated the possible cardiovascular effect of CnF in HEM. The groups were as follows: vehicle, cobalt chloride (CoCl$_2$, a reagent that blocks the activity of nucleus): microinjection of CoCl$_2$ (1mM) into the CnF; HEM: blood withdrawal of about 15% of TBV, Hem+saline, and HEM+CoCl$_2$ groups. In all groups, Blood Pressure (BP) and also Heart Rate (HR) were recorded by a PowerLab instrument and the changes were calculated and compared. The results indicated that HEM decreased BP and increased HR. However, these effects (decreased BP and increased HR) improved only after the inactivation of the CnF. Our results confirmed the involvement of CnF in cardiovascular regulation of BP and HR during HEM.

1. Introduction

Hemorrhage (HEM) is a life-threatening incident due to the loss of intravascular volume caused by hypotension with two compensatory and non-compensatory phases (Evans, Ventura, Dampney, & Ludbrook, 2001; Standl, Annecke, Cascorbi, Heller, Sabashnikov, & Teske, 2018). The compensatory phase is performed by the elicit of baroreceptors signaling hypotension to the Nucleus of the Solitary Tract (NTS) followed by the activation of vasomotor neurons in Rostral Ventrolateral Medulla (RVLM), an important area in cardiovascular modulation, that excites the sympathetic neurons and maintains blood pressure in normal condition (Naves et al., 2018; Palkovits, 1999). If blood loss continues, the non-compensatory phase, as the next phase, is started, in which sympathetic drive abruptly reduces and arterial pressure falls. The underlying mechanism responsible for these phases of HEM is not completely understood (Evans et al., 2001) and it has suggested that in addition to NTS (Buller, Smith, & Day, 1999) and RVLM (Dean & Bago, 2002), other nuclei also are involved. For example, it has reported that the Paraventricular Nucleus of the Hypothalamus (PVN) (Fan & McIntosh, 1994) and parabrachial areas (Blair & Mickelsen, 2006) are associated with the cardiovascular regulation during HEM.

Also, numerous nuclei, such as Cuneiform Nucleus (CnF) are found in the brain that play important role in cardiovascular regulation; however, their role in HEM condition has not yet defined (Verberne, 1995).

The CnF is a reticular nucleus in the midbrain located in the anterolateral of Periaqueductal Grey (PAG) matter and begins from caudate part of the midbrain and continues to the rostral part of pons (Gioia & Bianchi, 1987). The CnF is precipitated in several functions, including regulation of motor activity, pain and respiratory modulation, integration of sensory-motor movement related to pain, defense reactions, and sleep (Shafei, Nasimi, Alaei, Pourshanazari, & Hosseini, 2012; Xiang, Zhu, Guan, & Ye, 2013). The role of CnF in cardiovascular regulation has also been reported in previous studies. Verberne reported that electrical stimulation of CnF increased sympathetic vasomotor outflow and blood pressure (Verberne, 1995). The role of CnF in cardiovascular regulation has been evaluated (Shafei et al., 2012). For example, our previous study indicated that glutamate increased blood pressure (Nasimi, Shafei, & Alaei, 2012), and the cholinergic system decreased cardiovascular parameters (Shafei, Niazmand, Hosseini, & Daloee, 2013). Despite the role of CnF in cardiovascular regulation, we previously indicated that inactivation of this nucleus by Cobalt Chloride (CoCl$_2$) did not change basic cardiovascu-

252

Mohebbati, R., et al. (2020). CnF and Hemorrhagic Hypotension. *BCN, 11*(3), 251-260.

lar parameters (Shafei et al., 2012). Although CnF is not active in basal conditions, it may affect cardiovascular responses in conditions, such as defense reaction, exercise, pain, and HEM (Verberne, Lam, Owens, & Sartor, 1997). Similarly, i Ahlgren et al. reported that HEM increased Fos-Like Immunoreactivity (FLI), a marker of brain activity, in the CnF (Ahlgren, Porter, & Hayward, 2007). Also, the relationship between CnF and brain regions, such as RVLM (Verberne, 1995), NTS (Buller, Smith, & Day, 1999) and PGA mater (Netzer et al., 2011) that regulate cardiovascular response during HEM has been reported. Accordingly, it is conceivable that the CnF is involved in cardiovascular adjustment during HEM. Therefore, the present study was conducted to evaluate the possible role of CnF in cardiovascular regulation during HEM.

2. Metods

2.1. Animal and recording of blood pressure

In this experiment, 30 male Wistar rats were used. Animals anesthetized with urethane (1.5 g/kg, IP). A heating lamp was used to maintain a relatively constant body temperature of the animals. The left femoral artery also was catheterized with an angiocath filled with heparinized saline (60 u/ml) to record Blood Pressure (BP) and Heart Rate (HR) using a blood pressure transducer and a PowerLab system (ID instrument, Australia) (Shafei & Nasimi, 2011). In all groups, Systolic Blood Pressure (SBP), Mean Atrial Pressure (MAP), and HR were recorded throughout the trial period.

2.2. Surgical procedures

The rats were mounted on a stereotaxic apparatus (Stoelting, USA). The scalp was incised and the skull was leveled between the bregma and lambda, and a small hole drilled above the CnF in the skull. The CnF stereotaxic coordinates were -7.6 to -8.5 mm caudal to the bregma, -1.7 to -2.2 mm lateral to the midline suture, and -5.5 to -6.2 mm ventral to the bregma according to the Paxinos and Watson atlas (Paxinos & Watson 2005). $CoCl_2$ (1mM) (Shafei, Alaei, & Farrokhi, 2011) was unilaterally microinjected into the CnF by a single-barreled micropipette (35-45 μm in diameter). For injection, the micropipette was connected through a PE-10 tube to a manual microinjector (Harvard) and carefully introduced into the CnF and injection was done during 30 s. The volume of the injected drug or saline was 100-150 nl in all groups (Shafei, Nikyar, Hosseini, Niazmand, & Paseban, 2017). The study protocol

was approved by the Bioethics Committee of Mashhad University of Medical Sciences (Code: 931769).

2.3. Hemorrhage protocol

In groups subjected to hemorrhagic hypotension, the right femoral artery was cannulated by a polyethylene tube filled with heparinized saline (60 u/ml) to prevent clotting during blood withdrawal. Blood withdrawal was initiated 5 min after a microinjection of $CoCl_2$ into the CnF. Blood withdrawal was performed from the femoral artery intermittently at a rate of 1 ml/100 g body weight for 10 min (Dean & Bago, 2002). In this method, about 15% of the Total Blood Volume (TBV) was withdrawn. The TBV of the rats was calculated previously as follows: 0.06 ml/g×body weight+0.77. This volume could induce an intermediate hemorrhage suitable to evaluate the role of cardiovascular regulation during HEM (Ahlgren et al., 2007).

At the end of each experiment, the animals' brain was removed and 24 h after fixation with formalin 10%, serial sections (50-micron thickness) were prepared using a microtome, and the location of the injections verified based on the atlas of Paxinos and a light microscope (Shafei et al., 2012).

2.4. Drug

The urethane and $CoCl_2$ were provided by the Sigma-Co., USA. All drugs were dissolved in saline.

2.5. Animal groups

Animals were randomly divided into the five following groups (n=6 per group): 1. Vehicle: Microinjection of saline into the CnF; 2. Cobalt Chloride ($CoCl_2$): Microinjection of CoCl2 (1mM) into the CnF; 3. HEM: Blood withdrawal of about 15% of TBV; 4. Hem+saline: saline microinjection into the CnF 5 min before HEM; and 5. HEM+$CoCl_2$: $CoCl_2$ microinjection into the CnF 5 min before HEM.

2.6. Data analysis

Changes (Δ) in cardiovascular parameters (SBP, MAP, and HR) were calculated and expressed as mean±SEM. The ΔSBP, ΔMAP, and ΔHR parameters in all groups were computed at each 5 min and statistically compared with the saline and HEM groups (repeated measures ANOVA). Also, the peak ΔSBP, ΔMAP, and ΔHR of each group were separately calculated 30 min after HEM (40 min after initiation of HEM) and compared with peak changes of the vehicle and HEM groups (independent sample t-test). A $P< 0.05$ was considered significant.

Mohebbati, R., et al. (2020). CnF and Hemorrhagic Hypotension. *BCN, 11*(3), 251-260.

253

3. Results

3.1. Effect of the vehicle and CoCl₂ microinjection into the CnF on cardiovascular parameters in normotensive rats

In this study, vehicle (saline) and $CoCl_2$ were separately injected into the CnF. Injection of saline into the CnF did not significantly change SBP, MAP, and HR compared with pre-injection. Inactivation of the CnF by microinjection of $CoCl_2$ also did not significantly affect basal cardiovascular parameters than the saline group (Table 1 and Figure 1).

3.2. Effect of hemorrhage on cardiovascular parameters

In HEM, withdrawal of blood was done over 10 min and cardiovascular responses were evaluated 45 min later. Figure 2 indicates changes in blood pressure and HR during HEM. Time-course changes in the responses also were plotted in Figures 3-6. As can be seen, at the end of blood withdrawal (10 min after initiation of hemorrhage), MAP, SBP, and HR decreased and then slowly returned, and after 20 min, SBP and MAP stabilized below the baseline levels; however, HR showed an increase (Figure 2). ΔMAP and ΔSBP after blood withdrawal were significantly lower compared with the vehicle group over time (repeated measures ANOVA; $P<0.01$, n=6), whereas HR firstly decreased followed by a significant increase (repeated measures ANOVA; $P<0.01$, n=6).

Also, 30 min after blood withdrawal (40 min after initiation of blood withdrawal), the peak ΔMAP, ΔSBP, and ΔHR were calculated and compared with the vehicle group. Compared with vehicle group, ΔSBP (HEM: -27.2±2.8 mmHg vs. vehicle: -2.3±1.9 mmHg, $P<0.01$) and ΔMAP (HEM: -21.6±3.4 mmHg vs. vehicle: -1.4±1.6 mmHg, $P<0.01$) significantly reduced, whereas ΔHR (HEM: 64.6±6.24 mmHg vs. vehicle: -6.7±3.8; $P<0.001$; independent sample t-test, Figures 3 and 4) significantly increased.
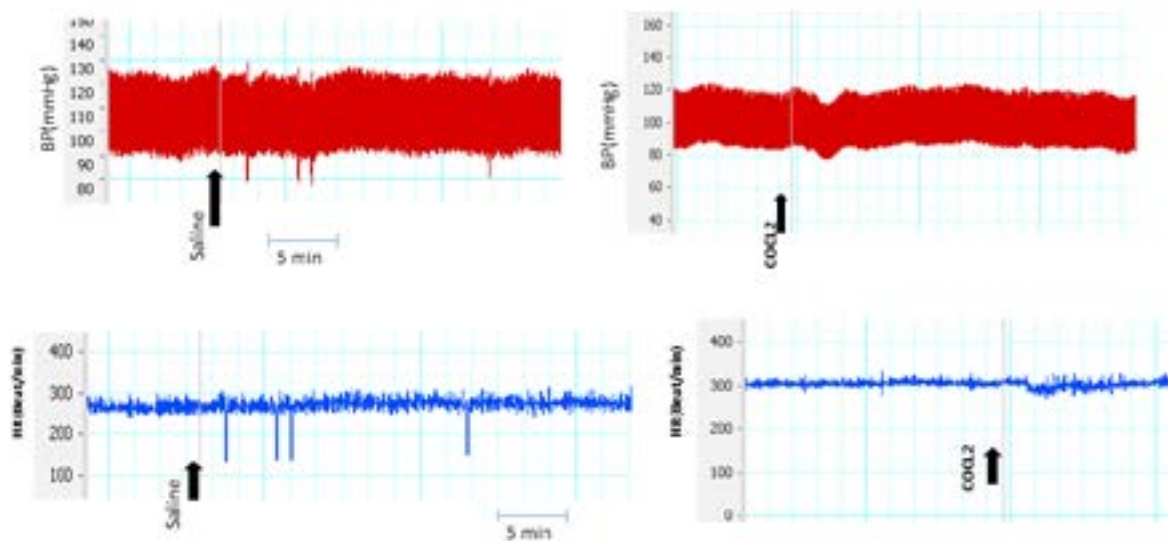

Figure 1. Recording Blood Pressure (BP) and Heart Rate (HR); Arrows indicate injection time.
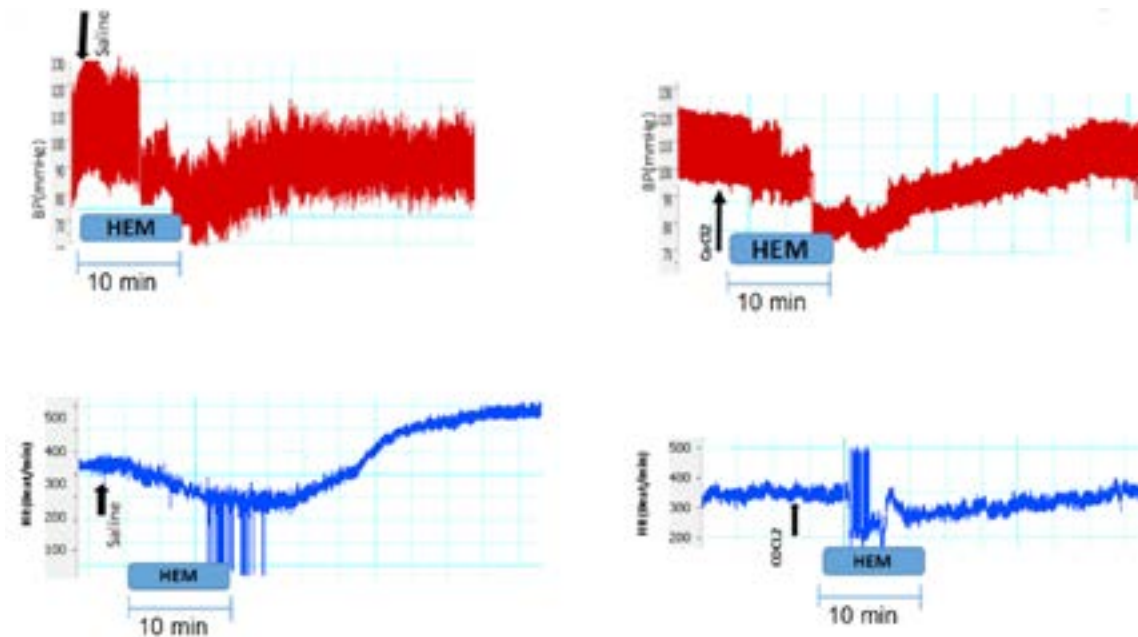
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Table 1. Cardiovascular effect of CoCl₂ and saline microinjection into the CnF

Group Parameter	SBP (mmHg)	MAP (mmHg)	HR (Beat/min)
Vehicle (saline)	124.2±2.3	113.5±1.9	330.6±7.3
CoCl₂	118.5±1.0	108.8±1.7	298±6.9

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Values are presented as Mean±SEM and an Independent sample t-test was used for data analysis; (n=6)

SBP: Systolic Blood Pressure; MAP: Mean Arterial Pressure; HR: Heart Rate; CnF: Cuneiform Nucleus; CoCl₂: Cobalt Chloride

Figure 2. Recording Blood Pressure (BP) and Heart Rate (HR) in the Hemorrhage (HEM) and HEM+ CoCl$_2$ groups; Arrows indicate injection time.

3.3. Inactivation of the CnF by CoCl$_2$ and its effect on cardiovascular responses in hypotension caused by hemorrhage

In this study, CnF was first inactivated by the microinjection of CoCl$_2$ and 5 min later, HEM performed over 10 min (Figure 3). Time-course changes of SBP, MAP, and HR in the HEM, HEM+saline, and HEM+CoCl$_2$ groups are shown in Figure 4. As can be seen, in the HEM+saline group, all parameters were not significant compared with the HEM group; however, they were
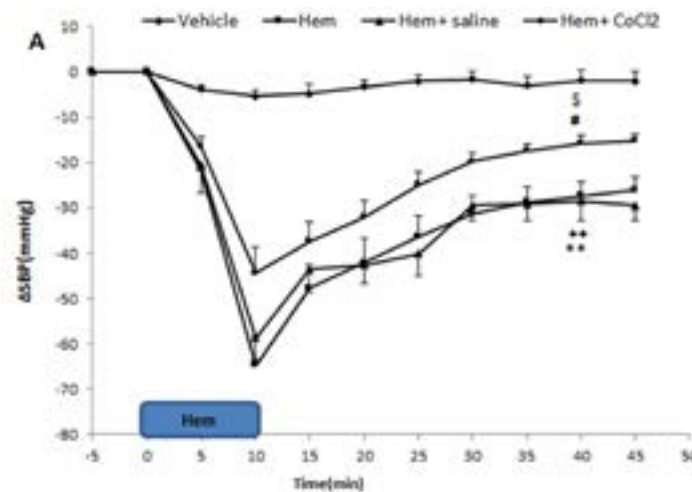
Figure 3. Time courses changes of Systolic Blood Pressure (ΔSBP) in the experimental groups

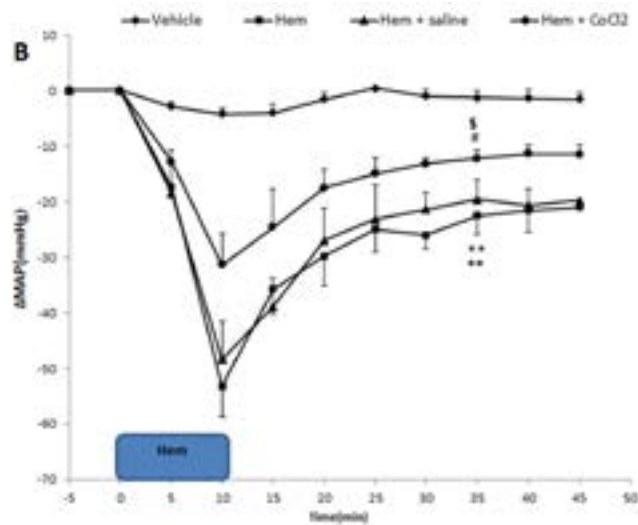HEM significantly decreased SBP than the vehicle group over time (P<0.01) and this effect was attenuated by cobalt chloride (CoCl$_2$), (repeated measures ANOVA, P<0.01). Values are expressed as Mean±SEM; (n=6).

Symbols indicated the peak ΔSBP, 30 min after blood withdrawal (independent sample t-test)

** P<0.001 HEM vs. vehicle group; $^{++}$P<0. 01 Hem+saline vs. vehicle group.

$^{#}$ P<0. 05 HEM+CoCl$_2$ vs. HEM group; $^{\$}$P<0.05 Hem+CoCl$_2$ vs. Vehicle group

Mohebbati, R., et al. (2020). CnF and Hemorrhagic Hypotension. *BCN, 11*(3), 251-260.

255

Figure 4. Time courses changes of Mean Arterial Pressure (ΔMAP) in the experimental groups

HEM significantly decreased ΔMAP than the vehicle group (P<0.01) and this effect was attenuated by cobalt chloride ($CoCl_2$) (repeated measures ANOVA, P<0.01).

Values are expressed as Mean±SEM; (n=6).

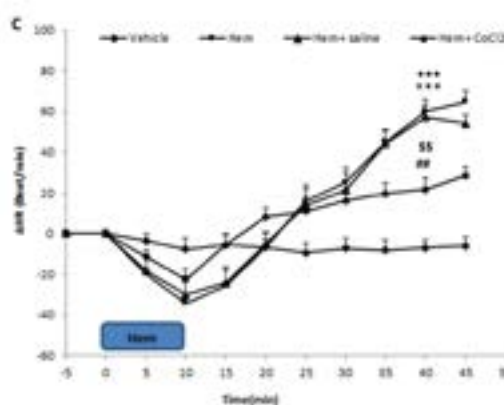Symbols indicated the peak ΔMAP, 30 min after blood withdrawal (Independent sample t-test)

**P<0. 01 HEM vs. vehicle group; [++] P<0. 01 HEM+saline vs. vehicle group

[#]P<0. 05 HEM+$CoCl_2$ vs. HEM group, [$] P<0.05: HEM+$CoCl_2$ vs. vehicle group

significant than the vehicle group (repeated measures ANOVA, P<0.01, Figure 4). In HEM+$CoCl_2$ group, both ΔMAP and ΔSBP significantly increased, whereas ΔHR significantly decreased than the HEM group (repeated measures ANOVA, P<0. 01, Figure 4).

The peak ΔSBP, ΔMAP, and ΔHR, 30 min after blood withdrawal in the HEM+saline and HEM+$CoCl_2$ groups were also evaluated. Comparing cardiovascular parameters 30 min after blood withdrawal (40th min) indicated that in the HEM+saline group, ΔSBP (-28.4±3.6 mmHg) and ΔMAP (-20.6±2.9 mmHg) were not significant

Figure 5. Time courses changes of ΔHR during HEM in the experimental groups.

HEM significantly increased than the vehicle group (P<0.001) and this effect was attenuated by $CoCl_2$ (repeated measures ANOVA, P<0.01). Values are expressed as Mean±SEM; (n=6).

Symbols indicate the peak ΔHR, 30 min after blood (independent sample t-test)

***P<0.001 HEM vs. vehicle group; [+++]P<0.001 HEM+saline vs. vehicle group

[##]P<0. 01 HEM+$CoCl_2$ vs. HEM group; [$$]P<0.01 HEM+$CoCl_2$ vs. vehicle group

256

Mohebbati, R., et al. (2020). CnF and Hemorrhagic Hypotension. *BCN, 11*(3), 251-260.
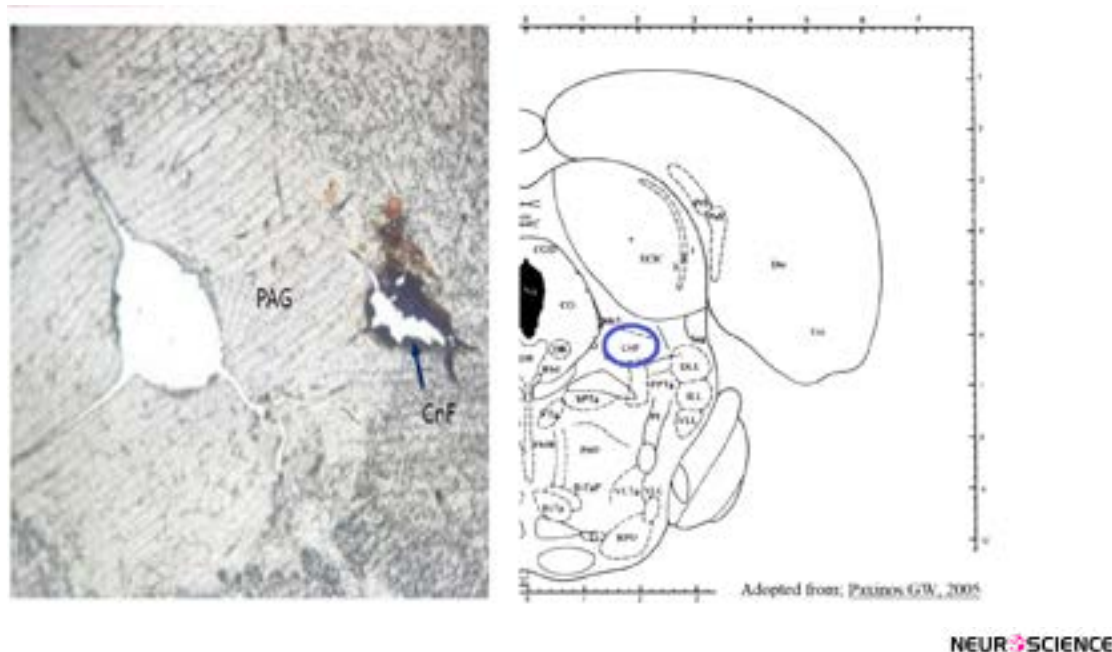
Figure 6. A Photomicrograph of a coronal brain section indicating the microinjection site in the Cuneiform Nucleus (CnF)

than the HEM group; however, they were lower than that of the vehicle group (-2.3±1.9 mmHg and -1.4±1.6 mmHg, respectively) (P<0.01, Figure 4). ΔHR in the HEM+saline group was significant in comparison with the vehicle group (54.3±5.6 beats/min vs. -6.7±3.8 beats/min, respectively; P<0.001, Figure 4).

In the HEM+CoCl$_2$ group, 30 min after termination of blood withdrawal, ΔSBP (-15.7±1.8 mmHg) and ΔMAP (12.2±1.4 mmHg) significantly increased than the HEM group (-27.2±2.8 mmHg and -21.6±3.4 mmHg, respectively); (P<0.05, Figure 4). In the HEM+CoCl2 group, ΔHR also significantly decreased compared with the HEM group (28.4±4.8 beats/min vs. 64.6±6.24 beats/min, respectively, P<0.01; Figure 4).

4. Discussion

We aimed at investigating whether the CnF is involved in returning the cardiovascular response to the normal level during hemorrhage. Therefore, the role of the CnF was studied in both normotensive and hemorrhagic hypotensive conditions. Our results indicated that in normotensive condition, inactivation of the CnF did not affect cardiovascular parameters. This effect confirms our previous experiment, in which CnF in the basal condition had no significant effect on the cardiovascular system (Shafei et al., 2012). Although CnF nucleus did not cause a cardiovascular response in basal condition; however, inactivation of this nucleus by CoCl$_2$ attenuated hypotension and tachycardia, induced by HEM. This effect

confirms the involvement of the CnF in cardiovascular response during HEM (Shafei et al., 2012). Our results are consistent with those reported by Ahlgren et al. that suggested the involvement of CnF in cardiovascular regulation during HEM. Algren et al. have shown that blood withdrawal of about 30% of TBV could initiate a non-compensatory phase and increase FLI in the CnF nucleus (Ahlgren et al., 2007).

Our experiment aimed to maintain the blood pressure of rats in the compensatory phase, i.e. blood withdrawal of 1 ml /100 g BW in 10 min. Loss of this volume of blood (about 15%) initially decreased both blood pressure and HR, and then HR significantly increased and blood pressure gradually returned and stabilized at about 30-40 mmHg lower than the baseline value. Reversible inactivation of CnF with CoCl$_2$ before HEM could attenuate low blood pressure and tachycardia induced by HEM. This effect showed that the CnF has inhibitory and excitatory effects on blood pressure and HR during HEM, respectively.

The mechanism (s) of this effect of the CnF is unclear; however, several mechanisms have been proposed. The presence of serotonergic, GABAergic, glutamatergic, cholinergic, and nitrergic neurons have been shown in the CnF (Gioia & Bianchi, 1987; Shafei & Nasimi, 2011; Shafei et al., 2013; Shafei et al., 2017; Verberne et al., 1997), of which cholinergic and nitrergic neurons have the inhibitory effect on the cardiovascular system (Shafei et al., 2013; Shafei et al., 2017). We suggest that in HEM,

Mohebbati, R., et al. (2020). CnF and Hemorrhagic Hypotension. *BCN, 11*(3), 251-260.

257

these neurons of the CnF are active and can delay the recovery of blood pressure. However, inactivation of the CnF nucleus by $CoCl_2$ (a synaptic blocker) blocked Ach release and accelerated the recovery of blood pressure. Other neurons present in the CnF and those involved in HEM have shown to be serotonergic neurons.

These neurons play an important role in the regulation of cardiovascular responses in normal and HEM conditions. For example, intracerebroventricular injection of serotonin increased both blood pressure and HR or 5-HT1A receptors of the NTS during HEM increased sympathetic activity. Serotonin neurons in CnF nucleus are associated with several functions CnF is involved, such as pain regulation. Currently, the role of CnF neurons in cardiovascular regulation in HEM is unknown and more studies are needed to indicate which neurotransmitter of the CnF is involved in this function (Gioia & Bianchi, 1987). Also, the relationship between CnF and RVLM, NTS, PVN, PAG, and Kölliker-Fuse nucleus (KF) areas has previously been reported (Korte, Jaarsma, Luiten, & Bohus, 1992).

The sympathoexcitatory effect of the CnF has shown to mostly be mediated by RVLM. However, the direct projection of CnF to RVLM is spare and indirect and mostly mediated by CnF-KF –RVLM pathway. Our previous study also indicated that the cardiovascular effect of the glutamatergic system of CnF is mediated via this pathway. As this pathway is excitatory and CnF has an inhibitory effect in HEM, it can be suggested that this pathway is not important in HEM. Also, CnF is associated with PAG and the cardiovascular effect of CnF is partly mediated by this Pathway. Due to the involvement of the PAG in the regulation of cardiovascular response during HEM, it is conceivable that this effect of the CnF can be mediated by the CnF-PAG pathway.

The CnF- raphe pathway can be another possible involved pathway. The relationship between CnF and raphe nuclei has been shown. Richter et al. indicated that this pathway plays a role in pain modulation (Richter & Behbehani, 1991). Therefore, it is possible that in addition to pain modulation, it could modulate cardiovascular response during HEM.

In HEM, chemo- and baroreceptors are active and can elicit chemo- and baroreflexes via projection to NTS. A projection from CnF to NTS has also been documented. Because NTS is an important area in the integration of chemo- and baroreflexes, it is conceivable that CnF via a relationship with NTS could modulate the activity of chemo- and baroreflexes. Also, Korte et al. indicated the connection of CnF with NTS and the dorsal motor nucleus of the vagus (Korte et al., 1992). Therefore, is can be suggested that the reduction of tachycardia induced by HEM after blockade of CnF with $CoCl_2$ is mediated by vagal system or NTS. In this study, CnF was inactivated unilaterally, which its effect can be different from bilateral inactivation. However, future studies are needed to evaluate the mechanism(s) of the CnF on cardiovascular regulation during HEM.

In conclusion, our results showed that CnF nucleus is involved in the adjustment of cardiovascular responses in HEM. Further studies should be conducted to evaluate the mechanisms and neural circuits of CnF involved in the regulation of cardiovascular response during HEM.

Ethical Considerations

Compliance with ethical guidelines

Funding

Authors' contributions

Conceptualization: Mohammad Naser Shafei and Mahmoud Hosseini; Methodology: Reza Mohebbati and Majid Khazaei; Investigation and writing-original draft: Reza Mohebbati; Writing-review & editing, supervision, and funding acquisition: Mohammad Naser Shafei; Resources: All author.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

References

Ahlgren, J., Porter, K., & Hayward, L. F. (2007). Hemodynamic responses and c-Fos changes associated with hypotensive hemorrhage: Standardizing a protocol for severe hemorrhage in

258

Mohebbati, R., et al. (2020). CnF and Hemorrhagic Hypotension. *BCN, 11*(3), 251-260.

conscious rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 292*(5), R1862-71. [DOI:10.1152/ajpregu.00325.2006] [PMID]

Blair, M. L., & Mickelsen, D. (2006). Activation of lateral parabrachial nucleus neurons restores blood pressure and sympathetic vasomotor drive after hypotensive hemorrhage. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 291*(3), R742-50. [DOI:10.1152/ajpregu.00049.2006] [PMID]

Buller, K. M., Smith, D. W., & Day, T. A. (1999). NTS catecholamine cell recruitment by hemorrhage and hypoxia. *NeuroReport, 10*(18), 3853-6. [DOI:10.1097/00001756-199912160-00024] [PMID]

Dean, C., & Bago, M. (2002). Renal sympathoinhibition mediated by 5-HT 1A receptors in the RVLM during severe hemorrhage in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 282*(1), R122-30. [DOI:10.1152/ajpregu.2002.282.1.R122] [PMID]

Evans, R. G., Ventura, S., Dampney, R. A. L., & Ludbrook, J. (2001). John Ludbrook APPS symposium neural mechanisms in the cardiovascular responses to acute central hypovolaemia. *Clinical and Experimental Pharmacology and Physiology, 28*(5-6), 479-87. [DOI:10.1046/j.1440-1681.2001.3473.x] [PMID]

Fan, L., & McIntosh, T. K. (1994). Effect of dynorphin microinjection in the paraventricular nucleus on the hemodynamic response to hemorrhage in the rat. *Circulatory Shock, 42*(4), 197-203. [PMID]

Gioia, M., & Bianchi, R. (1987). The cytoarchitecture of the nucleus cuneiformis. A Nissl and Golgi study. *Journal of Anatomy, 155*, 165-76. [PMID] [PMCID]

Korte, S. M., Jaarsma, D., Luiten, P. G. M., & Bohus, B. (1992). Mesencephalic cuneiform nucleus and its ascending and descending projections serve stress-related cardiovascular responses in the rat. *Journal of the Autonomic Nervous System, 41*(1-2), 157-76. [DOI:10.1016/0165-1838(92)90137-6]

Nasimi, A., Shafei, M. N., & Alaei, H. (2012). Glutamate injection into the cuneiform nucleus in rat, produces correlated single unit activities in the Kolliker-Fuse nucleus and cardiovascular responses. *Neuroscience, 223*, 439-46. [DOI:10.1016/j.neuroscience.2012.07.041] [PMID]

Naves, L. M., Marques, S. M., Mourão, A. A., Fajemiroye, J. O., Xavier, C. H., & de Castro, C. H., et al. (2018). Involvement of median preoptic nucleus and medullary noradrenergic neurons in cardiovascular and sympathetic responses of hemorrhagic rats. *Scientific Reports, 8*(1), 11276. [DOI:10.1038/s41598-018-29310-z] [PMID] [PMCID]

Netzer, F., Bernard, J. F., Verberne, A. J., Hamon, M., Camus, F., & Benoliel, J. J., et al. (2011). Brain circuits mediating baroreflex bradycardia inhibition in rats: An anatomical and functional link between the cuneiform nucleus and the periaqueductal grey. *The Journal of Physiology, 589*(Pt 8), 2079-91. [DOI:10.1113/jphysiol.2010.203737] [PMID] [PMCID]

Palkovits, M. (1999). Interconnections between the neuroendocrine hypothalamus and the central autonomic system: Geoffrey Harris Memorial Lecture, Kitakyushu, Japan, October 1998. *Frontiers in Neuroendocrinology, 20*(4), 270-95. [DOI:10.1006/frne.1999.0186] [PMID]

Paxinos G., & Watson, C. (2005). *The rat brain in stereotaxic coordinates*. Burlington, MA: Elsevier Science. https://books.google.com/books?id=LKJqAAAAMAAJ&dq

Richter, R. C., & Behbehani, M. M. (1991). Evidence for glutamic acid as a possible neurotransmitter between the mesencephalic nucleus cuneiformis and the medullary nucleus raphe magnus in the lightly anesthetized rat. *Brain Research, 544*(2), 279-86. [DOI:10.1016/0006-8993(91)90065-4]

Shafei, M. N., Alaei, H., & Farrokhi, E. (2011). Effect of reversible inactivation of the Kolliker fuse nucleus on basal blood pressure and heart rate in anesthetized rat. *Basic and Clinical Neuroscience, 3*(1), 4-8. http://bcn.iums.ac.ir/article-1-189-en.html

Shafei, M. N., & Nasimi, A. (2011). Effect of glutamate stimulation of the cuneiform nucleus on cardiovascular regulation in anesthetized rats: Role of the pontine Kolliker-Fuse nucleus. *Brain Research, 1385*, 135-43. [DOI:10.1016/j.brainres.2011.02.046] [PMID]

Shafei, M. N., Nasimi, A., Alaei, H., Pourshanazari, A. A., & Hosseini, M. (2012). Role of cuneiform nucleus in regulation of sympathetic vasomotor tone in rats. *Pathophysiology, 19*(3), 151-5. [DOI:10.1016/j.pathophys.2011.11.001] [PMID]

Shafei, M. N., Niazmand, S., Enayatfard, L., Hosseini, M., & Hasanzade Daloee, M. (2013). Pharmacological study of cholinergic system on cardiovascular regulation in the cuneiform nucleus of rat. *Neuroscience Letters, 549*, 12-7. [DOI:10.1016/j.neulet.2013.05.046] [PMID]

Shafei, M. N., Nikyar, T., Hosseini, M., Niazmand, S., & Paseban, M. (2017). Cardiovascular effects of nitrergic system of the pedunculopontine tegmental nucleus in anesthetized rats. *Iranian Journal of Basic Medical Sciences, 20*(7), 776-82. [DOI:10.22038/IJBMS.2017.9009] [PMID] [PMCID]

Standl, T., Annecke, T., Cascorbi, I., Heller, A. R., Sabashnikov, A., & Teske, W. (2018). The nomenclature, definition and distinction of types of shock. *Deutsches Ärzteblatt International, 115*(45), 757-68. [DOI:10.3238/arztebl.2018.0757] [PMID] [PMCID]

Verberne, A. J. (1995). Cuneiform nucleus stimulation produces activation of medullary sympathoexcitatory neurons in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 268*(3), R752-8. [DOI:10.1152/ajpregu.1995.268.3.R752] [PMID]

Verberne, A. J., Lam, W., Owens, N. C., & Sartor, D. (1997). Supramedullary modulation of sympathetic vasomotor function. *Clinical and Experimental Pharmacology and Physiology, 24*(9-10), 748-54. [DOI:10.1111/j.1440-1681.1997.tb02126.x] [PMID]

Xiang, H. B., Zhu, W. Z., Guan, X. H., & Ye, D. W. (2013). The cuneiform nucleus may be involved in the regulation of skeletal muscle tone by motor pathway: A virally mediated trans-synaptic tracing study in surgically sympathectomized mice. *Brain, 136*(Pt 10), e251. [DOI:10.1093/brain/awt123] [PMID]

Mohebbati, R., et al. (2020). CnF and Hemorrhagic Hypotension. *BCN, 11*(3), 251-260.

259

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