THE ROLE OF NON-NMDA RECEPTOR OF GLUTAMATE IN CUNEIFORM NUCLEUS ON CARDIOVASCULAR RESPONSE IN ANAESTHETIZED RATS

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Summary

Cuneiform nucleus (CnF) is a reticular area of the midbrain that involved in the cardiovascular functions. It was shown that electrical and chemical stimulation of the CnF caused an increase in arterial blood pressure and excitation of sympathetic vasomotor discharge. However, there is not any study on cardiovascular effects of the glutamate and its receptors subtypes in the CnF. In the present study we investigated the cardiovascular effects of glutamate and its non-NMDA receptor in the CnF of rat. Rats were anesthetized and instrumented with an arterial catheter. Blood pressure (BP) and heart rate (HR) were recorded throughout each experiment. The drugs (50nl) were microinjected into the CnF. The maximum change was compared with control and pre-injection. Unpaired *t* test and pair *t* test was used for data analysis.

Microinjection of glutamate into CnF produced two types of responses: 1) short pressor and bradycardic; 2) long pressor and tacycardic responses. Microinjection CNQX alone had no significant cardiovascular effect but coinjection of CNQX and glutamate has similar but with smaller attenuation compared to that of glutamate.

Cardiovascular effects of the CnF may be mediated through glutamate receptors, but the role of non-NMDA receptor is not important.

Keywords: CnF, Arterial pressure, Heart rate, Glutamate, CNQX

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Introduction

The cuneiform nucleus (CnF) is a reticular area of the midbrain that involved in central cardiovascular regulation (1, 2) and response to threatening or painful stimuli by defensive behavioral and cardiovascular changes (3, 4). The CnF also plays important role in pain modulation, sensory/motor integration relevant to pain transmission (5, 6), modulation of respiration rate and motor activity (7). Both chemical and electrical stimulation of the CnF evoked an increase in arterial blood pressure and excitation of the lumbar sympathetic vasomotor discharge (2, 4).

Neuroanatomical studies showed that CnF being connected with regions involved in cardiovascular regulation, such as the periaqueductal gray matter (PAG), the parabrachial/kolliker-fuse nuclei complex, locus ceruleus, nucleus of the solitary tract (NTS), rostral ventrolateral medulla (RVLM) and dorsal motor nucleus of the vagus (1, 4, 8).

Previous studies have been shown that cardiovascular effect of the CnF is due to activation of the RVLM neurons (1-4, 8). Neurons in the RVLM directly innervate the preganglionic sympathetic neurons in the intermediolateral column of the spinal cord and play a critical role in the sympathetic tone and blood pressure (9, 10).

Glutamate via ionotropic and the metabotropic receptors plays a critical role in regulation of central cardiovascular system (11). The ionotropic receptors are further divided into NMDA (N-methyl-d-aspartic acid) and non-NMDA (kainate and amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA)) receptor-subtypes (11, 12). The glutamate receptor has been shown in CnF (5). But the cardiovascular roles of the glutamate and their receptors subtypes have not yet been studied in the CnF.

The present study was designed to evaluate the effects of non-NMDA receptor on mean arterial blood pressure and heart rate responses in the CnF nucleus of anesthetized rats.

Materials and methods

Animal preparation

Experiments were performed on thirty male Wistar rats weighing 250–320 g (Razi Institute, Tehran, Iran). Animals were housed individually in plastic cages with free access to food and water and under a 12-h light/dark cycle.

The rats were anesthetized with urethane (1.4 g/kg, ip), and supplementary doses (0.7 g/kg) were given if it was necessary until completion of the experiment.

The trachea was intubated for artificial ventilation. Temperature was kept at 37.5^{oC}with a thermostatically controlled heating pad. After induction of anesthesia a polyethylene catheter (PE-50) filled with heparinized saline were placed into the left femoral artery for Blood pressure (BP) and heart rate (HR) measurement. The BP and HR were continuously recorded by both a Harvard polygraph and a computer program written in this laboratory.

Drugs

The drugs used in the experiments were: urethane (Sigma, USA), L-glutamate (Sigma, USA) and CNQX (6-cyano-7-nitroquinoxaline-2-3-dione, an AMPA receptor antagonist, Sigma). L-glutamate was dissolved in normal saline. CNQX was first solubilized with dimethyl sulfoxid (DMSO) (Aldrich, USA) then dissolved in normal saline (0.9% NaCl).

Microinjections

Drug microinjections into CnF were performed by a single barreled micropipette with internal diameter ranging 35–45 μm according to procedure previously described. The animals were placed in a stereotaxic apparatus (Stoelting, USA). The scalp was longitudinally incised and skull was leveled between lambda and bregma and a small hole drilled in the skull.

The stereotaxic coordinates of CnF were 7.6–8.5 mm caudal to bregma, 1.7–2.2 mm lateral to the midline suture and 5.5–6.2 mm ventral from the bergma according to the atlas of Paxinos and Watson (13). The micropipette connected through PE-10 tube to an injection syringe and was carefully introduced into the CnF and injection was done in two sites with 300 μm apart. The interval between injections was 20 min.

Experimental groups

To examine cardiovascular effects of non-NMDA receptor of glutamatergic system on CnF the following groups used:

– The control group; which vehicle (normal saline) was injected into CnF
– Injection of 50 nl of L-glutamate (0.25 M, Sigma).
– Injection of 50nl of (1mM) of CNQX (6-cyano-7-nitroquinoxaline-2-3-dione, an AMPA/kinat receptor antagonist, Sigma).
– Co-injection of 50nl of L-glutamate (0.25 M) + CNQX (1mM).

Then values given in the results are the number of injections and not the number of individual animals.

Histological procedure

At the end of each experiment, the rats were anesthetized with high dose of the urethane, chest was surgically opened and the brain perfuses transcardially with 100 ml of 0.9% saline, followed by 100ml of 10% formalin. The brain was removed and stored in 10% formalin for at least 24h at 4^{oC}. Frozen serial transverse sections (50μm) of brain stem were cut using a cryostat at -20^{oC}. Brain sections were stained with cresyl violet 1% and the injection sites were determined according to the rat brain atlas of Paxinos and Watson under the light microscope (14, 15).

Data analysis

The data of the blood pressure and heart rate was expressed as mean ± SEM. The course of changes in the heart rate and arterial pressure was plotted. The maximum change was compared with the pre-injection (paired *t*-test) and control (un paired *t*-test) values. The criterion for a statistical significance was a $P<0.05$.

Results

Baseline values of mean blood pressure (MAP) and heart rate (HR) were 93.56±2.50 mmHg and 343±8.96 beats /min, respectively. Microinjection of saline into the CnF did not produced significant changes in baseline MAP and HR (Fig 1). In glutamate group, baseline MAP was 91.21±5.34mmHg and HR was 378.54±38.66 beats/min. Microinjection of glutamate (0.25 M, 50-100 nl) produced two distinct short and long responses. In short response, immediately after injection MAP increased, while HR decreased. Time course changes of these effects have been shown in Fig.1. As shown in this diagram administration of glutamate elicited significantly increased in MAP (unpaired *t* test $p<0.001$, n=16) and caused significantly decreased in HR (unpair *t* test $p<0.01$ n=16) compare to control group. The maximal change for MAP was 13.2 ± 1.7 mmHg (pair *t* test $p<0.001$) and for HR was -21.6 ± 8.9 beats /min. (pair *t* test, $p<0.01$).

In long response both MAP and HR increased slowly and reached a peak within 2 minute and remained upper baseline for approximately 8 minute. In this response MAP (unpaired *t* test $P<0.001$ n=11) and HR (unpaired *t* test $P<0.01$, n=11) significantly increased compare to control. Time course of these changes in long response are shown in Fig .2. The maximal change for MAP was 19.2± 2.09 mmHg (pair *t* test $p<0.001$) and for HR was 21.62± 11.90 beats /min (pair *t* test $p<0.05$).

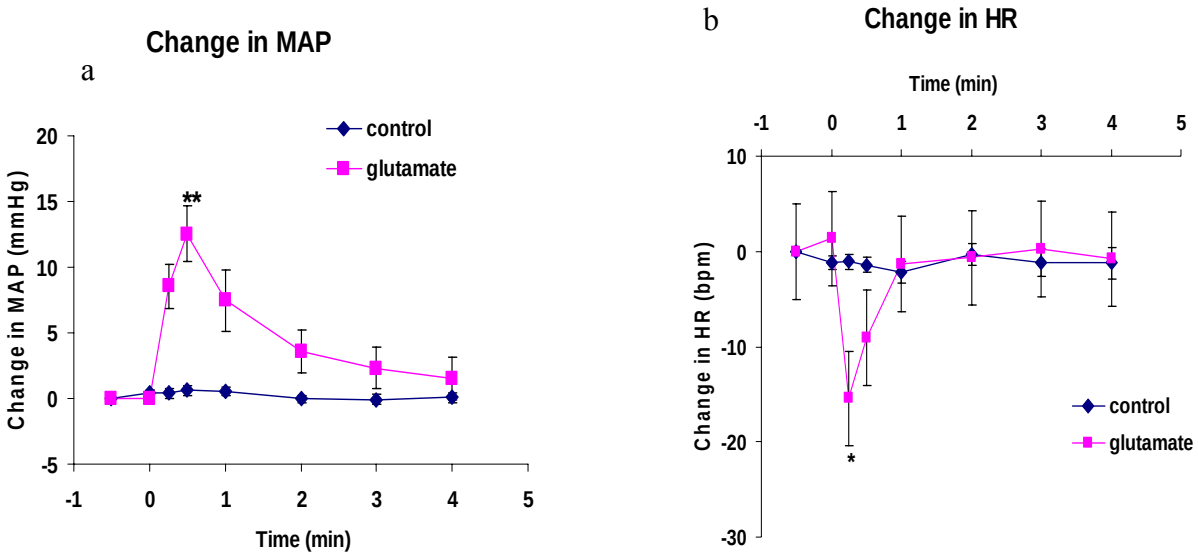

Fig.1. Short Cardiovascular effects to microinjection of glutamate into the CnF (n=16) compared to the control group (n=21). Time courses of changes in MAP (a) and HR (b). Glutamate significantly increased MAP (un pair *t*-test; P<0.001) and decreased HR (un pair *t*-test; P<0. 01). Values are presented as mean± SEM. *P < 0.01; **P < 0.001 maximal changes compare with pre- injection value (paired *t* test)

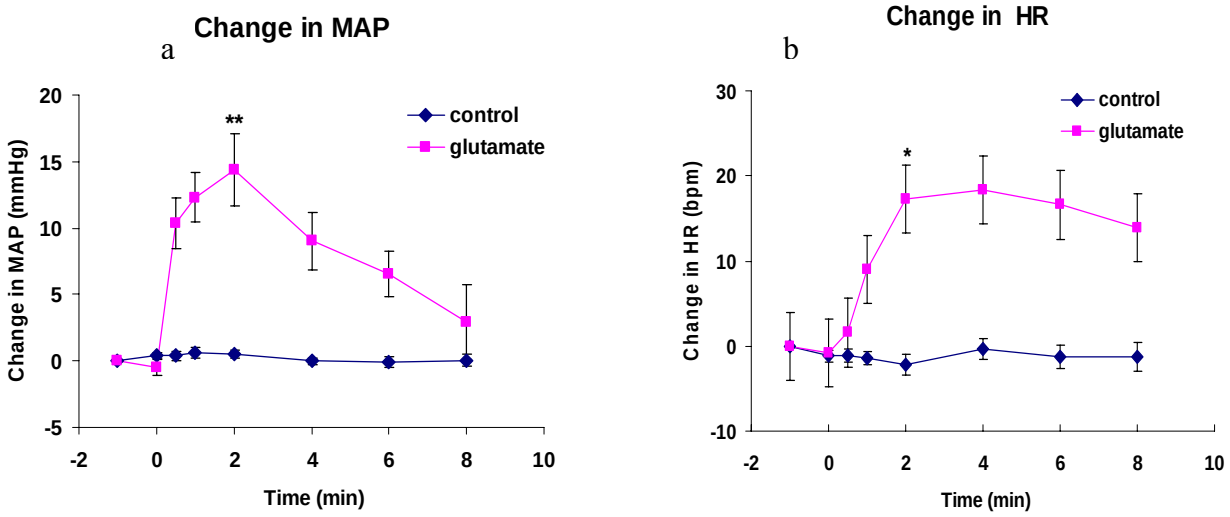

Fig.2. Long cardiovascular effects of microinjection of glutamate into the CnF (n=11) compared to the control group (n=21). Time courses of changes in MAP (a) and HR (b).
Glutamate significantly increased MAP and HR (unpair *t* test; P<0.001, P<0.01 respectively). Values are presented as mean± SEM.
*P < 0.01; **P < 0.001 maximal changes compare with pre- injection value (paired *t* test)

To determine if the response of glutamate was mediated through non-NMDA receptor subtype, we microinjected CNQX (AMPA/kinat receptor antagonist) in the CnF. Microinjection the CNQX alone can not altered any changes in MAP and HR. To make sure of the presence this subtype receptor in the CnF, we co-injected CNQX with glutamate in another group.

Co-injected of glutamate (0.25 M) +CNQX (1mM n=10) also evoked two short and long responses. However, these responses were lower than the glutamate effect (Fig 3, 4).

In short responses of glutamate + CNQX group, the peak changes of MAP and HR were lower than those of the glutamate group but the changes were not significant (ΔMAP: 10.9±2mmHg vs.13.2±1.7 mmHg; ΔHR: -22.7 ±12.5 beats /min vs. -21.6±8.9 beats/min, n=11) (Fig. 3).

In long responses coinjection of glutamate and CNQX increased both MAP and HR. The peak changes of MAP and HR were lower than those of the glutamate group. However, only change in MAP was significant (ΔMAP: 9.5±1.9 mmHg vs. 19.2±2.0 mmHg, *t*-test, P <0. 01; ΔHR: 3.60± 14.98 beats /min vs. 21.6±11.9 beats /min, n=6) (Fig. 4).

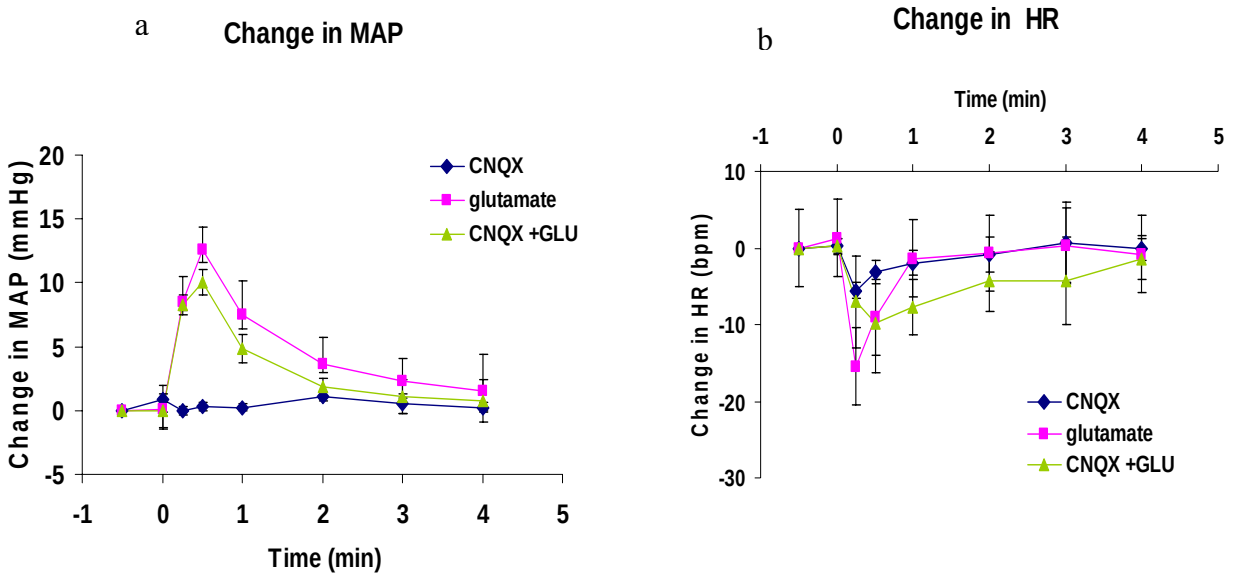

Fig.3. Short responses to coinjection of glutamate + CNQX into the CnF (n=10) compared to the glutamate (n=16) group. Time courses of changes in MAP (a) and HR (b). Glutamate + CNQX increased MAP and decreased HR. There is no significant difference between glutamate and glutamate + CNQX groups

Discussion

The result of present study showed that microinjection of glutamate into the CnF elicited two short and long responses. In short response glutamate caused pressor and bradycardic effects and in long response elicited pressor and tachycardic effects. These findings are similar with the results of the previous study, which showed that electrical stimulation of CnF evoked increased MAP and bimodal, short and long, sympathoexcitatory response. This result suggested that different pathways are involved in transmitting signals from the CnF to spinal cord (2, 4).

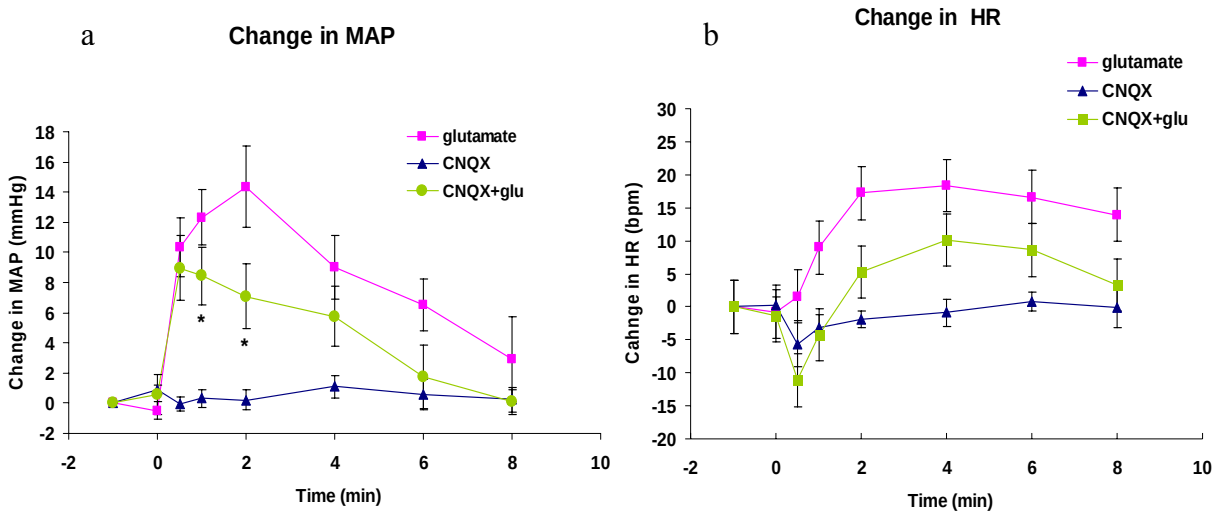

Fig.4. Long responses to microinjection of glutamate + CNQX into the CnF (n=8) compared to the glutamate (n=9) group. Time courses of changes in MAP (a) and HR (b). Glutamate + CNQX increased both MAP and HR. Only change in MAP was significant. *(*unpair *t* test; P<0.01)
Values are presented as mean± SEM. * P < 0.01

Electrical stimulation of the CnF caused an increase in MAP with activation of sympathoexcitatory neurons in RVLM. These results are shown that the CnF is a sympathoexcitatory nucleus and its cardiovascular effects in part, are due to excitation of premotor sympathoexcitatory neurons in RVLM (1-3). RVLM has two major population neurons with different conduction velocity (3, 4). Therefore, it has been postulated that bimodal response of the CnF as result of activation of this two population neurons.
It has been shown that the CnF projects to the PAG .The PAG also involved in central cardiovascular regulation(4).So, PAG is another area for relaying cardiovascular response of CnF. In addition, our study showed bradycardic and tachycardic responses. These effects suggested the coexistence of two opposite cardiovascular mechanisms in regulation of HR in the CnF. The projection from CnF to forebrain area and paraberachial nucleus, kolliker-fuse and RVLM maybe caused pressor and tachycardic response and, descending fibers to the motor nucleus of the vagus and nucleus tractus solitarius are parasympathetic limb and involved in the bradycardiac response (1, 16).
To examine the effects of non-NMDA of glutamate receptor, CNQX(an AMPA/kinat receptor antagonist) was microinjected into the CnF. Blockade of the AMPA/kinat receptors alone did not elicit statistically significant changes in the MAP or HR, indicating that under our experimental condition, there was no or little release of glutamate in the CnF.
Based on this finding it could be postulated that at normal condition, glutamatergic neurons of the CnF are not active and a drop of the arterial pressure or other stresses such as pain might activate them (14).
Since blockade of the receptors alone did not produce a significant effect, we co injected glutamate+ CNQX to make sure of the presence of glutamate in the injection sites. Coinjection of glutamate with CNQX attenuated the cardiovascular responses elicited by glutamate. However, this effect was not significant. This finding showed that the role of AMPA/kinat receptors is not necessary for cardiovascular effects of glutamate in CnF.

Previous studies showed that the short response of electrical stimulation of CnF mediated by fast conducting neurons and was significantly blocked by intrathecal kynurenic acid (2-4). But short response in our study can not blocked by AMPA/kinat antagonists. Therefore, it is possible that short response mediated through NMDA receptor. In addition, Lam et al (1997) showed that long latency effect of CnF was partly reduced by intrathecal administration of Prazosin, a selective α_1-adrenoceptor antagonist and methiothepin, a non-selective serotonin receptor antagonist (2). This result showed that long response probably mediated by slow-conducting catecholaminergic and serotonergic neurons. However, exact mechanisms of these effects are not known.

The CnF is considered as a part of the pain modulation system and its antinociceptive effects is mediated, in part, by glutamate receptors at the level of raphe magnus nucleus (5, 17 and 18). It is well known that pain and other peripheral stimulation evoked cardiovascular response, such as increased in MAP, HR and myocardial contractility (19, 20). Several studies showed that PAG, raphe nuclei and paraberachial nucleus are important sites for integration of pain and cardiovascular (20). In addition glutamate play critical role in the integration cardiovascular during nociception (20, 21). Because CnF has projection to cardiovascular and pain centers, it is possible that glutamatergic system of the CnF is involved in integration of cardiovascular and pain responses. However, further investigations are needed to elucidate the actual role of CnF in these effects.

In summary, our results showed that chemical stimulation of CnF with glutamate caused short and long pressor with bradycardic and tachycardic responses, respectively. These results showed that cardiovascular effect of the CnF is mediated mainly through glutamate. But the role of the non-NMDA receptor subtype is not important.

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References

1. Korte SM, Jaarsma D, Luiten PG, Bohus B. Mesencephalic cuneiform nucleus and its ascending and descending projections serve stress-related cardiovascular responses in the rat. J Auton Nerv Syst, 1992 ; 41:157 -76.
2. Lam W, Verberne AJ,Cuneiform nucleus stimulation-induced sympathoexcitation: role of adrenoceptors, excitatory amino acid and serotonin receptors in rat spinal cord, Brain Res, 1997 May 23;757(2):191-2.
3. Verberne AJ,Cuneiform nucleus stimulation produces activation of medullary sympathoexcitatory neurons in rats, Am J Physiol, 1995; 268:R752-8.
4. Verberne AJ, Lam W, Owens NC, Sartor D.Supramedullary modulation of sympathetic vasomotor function. Clin Exp Pharmacol Physiol. 1997; 24 (9-10):748-54. Review
5. Haghparast A, Gheitasi IP, Lashgari R.Involvement of glutamatergic receptors in the nucleus cuneiformis in modulating morphine-induced antinociception in rats. Eur J Pain. 2007; 11(8):855-62.
6. Zemlan FP, Behbehani MM. Nucleus cuneiformis and pain modulation: anatomy and behavioral pharmacology. Brain Res 1988 Jun 21; 453(1-2):89-102.
7. Bringmann A, Klingberg F.Electrical stimulations of the basal forebrain and the nucleus cuneiformis differently modulate behavioural activation of freely moving rat. Biomed Biochim Acta.1989; 48 (10):781-91.

8. Lam W, Gundlach AL, Verberne AJ. Increased nerve growth factor inducible-A gene and c-fos messenger RNA levels in the rat midbrain and hindbrain associated with the cardiovascular response to electrical stimulation of the mesencephalic cuneiform nucleus. Neuroscience 1996 Mar; 71(1):193-211.
9. Guyenet PG .The sympathetic control of blood pressure. Nat Rev Neurosci 2006 May; 7(5):335-46. Review
10. Dampney RA, Horiuchi J, Tagawa T, Fontes MA, Potts PD, Polson JW. Medullary and supramedullary mechanisms regulating sympathetic vasomotor tone. Acta Physiol Scand 2003; 177(3):209-18. Review
11. Karlsson GA, Preuss CV, Chaitoff KA, Maher TJ, Ally A. Medullary monoamines and NMDA-receptor regulation of cardiovascular responses during peripheral nociceptive stimuli. Neurosci Res. 2006 Jul; 55(3):316-26.
12. Hatam M, Nasimi A. Glutamatergic systems in the bed nucleus of the stria terminalis, effects on cardiovascular system. Exp Brain Res. 2007 Apr; 178(3):394-401.
13. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 5th ed. San Diego: Elsevier Academic Press; 2005, p. 98–106.
14. Resstel LB, Fernandes KB, Corrêa FM .Medial prefrontal cortex modulation of the baroreflex parasympathetic component in the rat. Brain Res. 2004 Jul 23; 1015(1-2):136-44.
15. Nasimi A, Hatam M.GABA and glutamate receptors in the horizontal limb of diagonal band of Broca (hDB): effects on cardiovascular regulation.Exp Brain Res. 2005 Nov; 167(2):268-75.
16. Bohus B, Koolhaas JM, Korte SM, Roozendaal B, Wiersma A. Forebrain pathways and their behavioural interactions with neuroendocrine and cardiovascular function in the rat. Clin Exp Pharmacol Physiol. 1996 Feb; 23(2):177-82.
17. Richter RC, Behbehani MM. 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 Res. 1991 Mar 29; 544 (2):279-86.
18. Zemlan FP, Behbehani MM.Nucleus cuneiformis and pain modulation: anatomy and behavioral pharmacology.Brain Res. 1988 Jun 21; 453(1-2):89-102.
19. Siddall PJ, Polson J.W and. Dampney RA.Descending antinociceptive pathway from the rostral ventrolateral medulla: a correlative anatomical and physiological study. Brain Res. 1994; 645: (61–68.
20. Karlsson GA, Preuss CV, Chaitoff KA, Maher TJ, Ally A. Medullary monoamines and NMDA-receptor regulation of cardiovascular responses during peripheral nociceptive stimuli.Neurosci Res. 2006 Jul;55(3):316-26.
21. Javanmardi K, Parviz M, Sadr SS, Keshavarz M, Minaii B and Dehpour AR. Involvement of N-methyl-d-aspartate receptors and nitric oxide in the rostral ventrolateral medulla in modulating morphine pain-inhibitory signals from the periaqueductal grey matter in rats. Clin. Exp. Pharmacol. Physiol. 2005; 32 : 585 –589.