Effect of Reversible Inactivation of the Kolliker Fuse Nucleus on Basal Blood Pressure and Heart Rate in Anesthetized Rat

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Article info:
Received: 2 August 2011
First Revision: 16 August 2011
Accepted: 5 September 2011

Key Words:
Kolliker-Fuse nucleus,
CoCl2,
Blood Pressure,
Heart Rate,
Reversible Inactivation.

ABSTRACT

Introduction: Several supra spinal areas such as rostral ventrolateral medulla (RVLM) area are involved in basic cardiovascular regulation. The Kolliker—Fuse nucleus (KF) is located in pons and is heavily connected with RVLM. The cardiovascular effect of KF nucleus has been shown and it is suggested that KF is involved in sympathetic vasomotor tone and basic cardiovascular regulation. Therefore, in the present study, the effects of KF on basic cardiovascular values were evaluated.

Methods: After induction of anesthesia, a polyethylene catheter (PE-50) filled with heparinized saline was inserted into the femoral artery of rats. Animals were then placed in a stereotaxic apparatus and KF nucleus was inactivated by microinjection of cobalt chloride (CoCl2). Blood pressure and heart rate (HR) were continuously recorded pre and post inactivation.

Results: Our result showed that inactivation of KF slightly changed mean arterial blood pressure (MAP) (92.3 ± 2.45 mmHg vs. 90.86 ± 1.7 mmHg) and HR (343.8 ± 4.6 beats/min vs. 350.7 ± 8.32 beats /min). However, these effects were not significant in comparison to the control group.

Discussion: We concluded that synapses in the KF nucleus have no effect on regulation of basal blood pressure and heart rate, because CoCl2 is a synaptic blocker.

1. Introduction

Vasomotor tone is a partial contraction for vascular blood vessels under resting condition which allows the central nervous system to respond to several challenges such as hemorrhage, exercise and changes in position (Dampney, Horiuchi, & Tagawa, 2003). The autonomic nervous system, especially the sympathetic nervous system has critical effect on vasomotor tone and heart rate. Sympathetic system contains pre and post ganglionic neurons. Pre ganglionic neurons are located in intermediolateral cell column (IML) of the spinal cord (Dampney, 1994; Dampney & Horiuchi, 2003; Dampney et al., 2003). Vasomotor activity and heart rate in resting conditions, heavily depend on inputs originating from several supra spinal areas such as rostral ventrolateral medulla area (RVLM), caudal pressor area (CPA) and paraventricular nucleus of the hypothalamus (PVN) (Campos et al., 2008; Dampney et al., 2003; Guyenet, 2006).

Among these areas, RVLM is a well known pressor region, playing a major role in the tonic and reflex control of sympathetic vasomotor activity and cardiovascular regulation (Guyenet, 2006; Guyenet, Haselton, &

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Sun, 1989).The RVLM receives several inhibitory and excitatory inputs from peripheral receptors and different nuclei in brain and also sends projections to many areas involved in cardiovascular regulation (Dampney, 1994; Dampneyet al., 2003; Guyenet, 2006). The parabrachial /Kolliker-Fuse (PB/KF) complex is an area that projects to RVLM. This complex is subdivided into three well-defined areas including: the medial parabrachial nucleus, the lateral parabrachial nucleus, and Kolliker Fuse nucleus (KF) (Fulwiler & Saper, 1984). The KF nucleus is an important part of this complex that has numerous functions such as control of respiratory system, pain modulation and regulation of cardiovascular system (Dutschmann, Morschel, & Kron, 2004). Electrical stimulation of the KF causes a pressor effect with mild tachycardia (Korte, Jaarsma, & Luiten, 1992). In addition, stimulation of cardiac sympathetic afferents evokes large numbers of neurons in the KF nucleus (Dutschmann & Herbert, 1997; Lam, Gundlach, & Verberne, 1996). The KF nucleus is also involved in relaying of cardiovascular responses of cuneiform nucleus (CnF) (Korte et al., 1992; Shafei & Nasimi, 2011). The relationship of KF nucleus with brain areas involved in cardiovascular regulation such as RVLM, nucleus tractus solitarius (NTS), CnF, raphe nucleus, periaqueductal grey (PAG) and the intermediolateral (IML) column in spinal cord has been also shown by anatomical research (Dampney & Horiuchi, 2003; Korte et al., 1992; Shafei & Nasimi, 2011). Although these evidences indicate the involvement of KF nucleus in cardiovascular regulation, there has been no study till now evaluating the effect of the KF nucleus on basal vasomotor tone. Therefore, in this study the effect of KF nucleus on vasomotor tone was studied. The KF nucleus was reversibly inactivated by cobalt chloride (CoCl2) (a nonselective synapse blocker) and its effects on basal mean arterial blood pressure (MAP) and heart rate (HR) was evaluated in anesthetized rats.

2. Methods

2.1. Animals and Surgery

Experiments in this study were carried out on 24 male Wistar rats (250–320 g). The rats were anesthetized intraperitoneally with urethane (1.4 g/kg), and supplementary doses (0.7 g/kg). The temperature was kept at 37.5C with a thermostatically controlled heating pad.

After induction of anesthesia, a polyethylene catheter (PE-50) filled with heparinized saline was inserted in the femoral artery for recording of blood pressure and heart rate. The BP and HR were continuously recorded by both a Harvard polygraph and a computer program written by Dr Ali Nasimi in department of physiology, Isfahan University of Medical Sciences.

2. 2. Stereotaxic Surgery and Drug Microinjection

After cannulation of artery, the animals were placed in a stereotaxic apparatus (Stoelting, USA). The scalp was incised and skull was leveled between lambda and bregma and a small hole drilled in the skull. The stereotaxic coordinates of KF were -8.4 to -8.8 mm caudal to bregma, -2.4 to -2.8 mm lateral to the midline suture and -7.3 to -7.8 mm ventral from the bergma according to the atlas of Paxinos and Watson (2005). Microinjection of the drug into the KF nucleus was done by a single barreled micropipette with internal diameter ranging 35–45 µm. The micropipette was connected through PE-10 tube to an injection syringe and was carefully introduced into the CnF and injection was done (Shafei & Nasimi, 2011).

2.3. Experimental Groups

The following groups were used:

. The control group; Injection of 100 nl of saline into the KF nucleus

. The CoCl2 group; Injection of 100 nl of cobalt chloride (CoCl2, 1mM, sigma) into the KF nucleus

2.4. Histological Procedure

At the end of each experiment, animals were transcardially perfused with 100 ml of 0.9% normal saline followed by 100 ml of 10% formalin. The brain was removed and stored in 10% formalin for at least 24h at 4oC. Frozen serial transverse sections (50µm) of brain stem were cut using a cryostat at -20oC. Brain sections were stained with cresyl violet 1% and the injection sites were verified according to the rat brain atlas of Paxinos and Watson (2005) by light microscope (Hatam & Nasimi, 2007).

2.5. Data Analysis

The data obtained from the blood pressure and heart rate was expressed as mean ± SEM. The time 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 (unpaired t-test) values. The criterion for a statistical significance was $P<0.05$.

3. Results

3.1 Effect of Saline Microinjection into the KF on Basal Mean Arterial Blood Pressure and Heart Rate in Anesthetized Rats

In this group, animals received saline (100 nl, n=8) into the KF nucleus. Injection of the saline did not significantly alter the mean arterial blood pressure (MAP; before: 90.3 ± 3.6 vs. after: 87.58 ± 2.76) and heart rate (HR; before: 332.28 ± 7. 84 vs. after: 345. 5 ± 6.72).

3.2. Effect of CoCl2 Microinjections into the KF on Basal Mean Arterial Blood Pressure and Heart Rate in Anesthetized Rats

In order to investigate the effect of KF nucleus on vasomotor tone, we inactivated this nucleus by microinjection of CoCl2 (1mM, 100 nl). A sample of the arterial pressure and heart rate tracings before and after the injection are shown in Fig. 1. Inactivation of KF nucleus slightly decreased mean arterial blood pressure (92.3 ± 2.45 mmHg vs. 90.86 ± 1.7 mmHg) and increased HR (343.8 ± 4.6 beats/min vs. 350.7 ± 8.32 beats /min). However, this effects are not significant compare to the control group and pre injection values (t-test, P >0.05, n = 10, Fig. 2). Time course of the changes in MAP and HR is shown in Fig. 3.

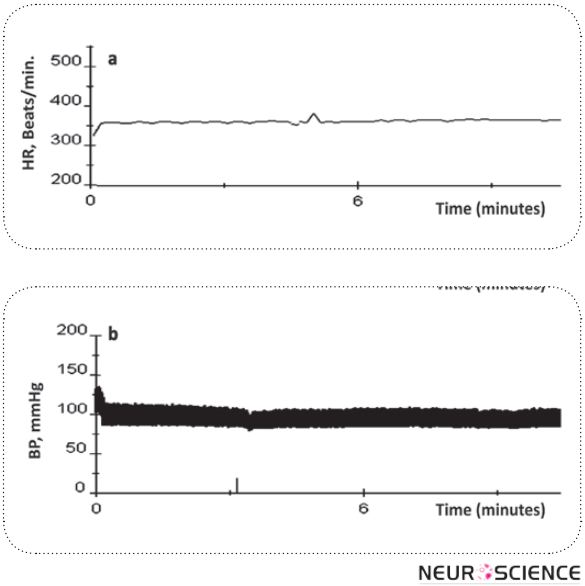

Figure 1. A typical tracing, showing the effects of microinjection of CoCl2 (1mM in100 nl) into the KF nucleus on (a) heart rate and (b) blood pressure. The vertical lines show the time of injection.

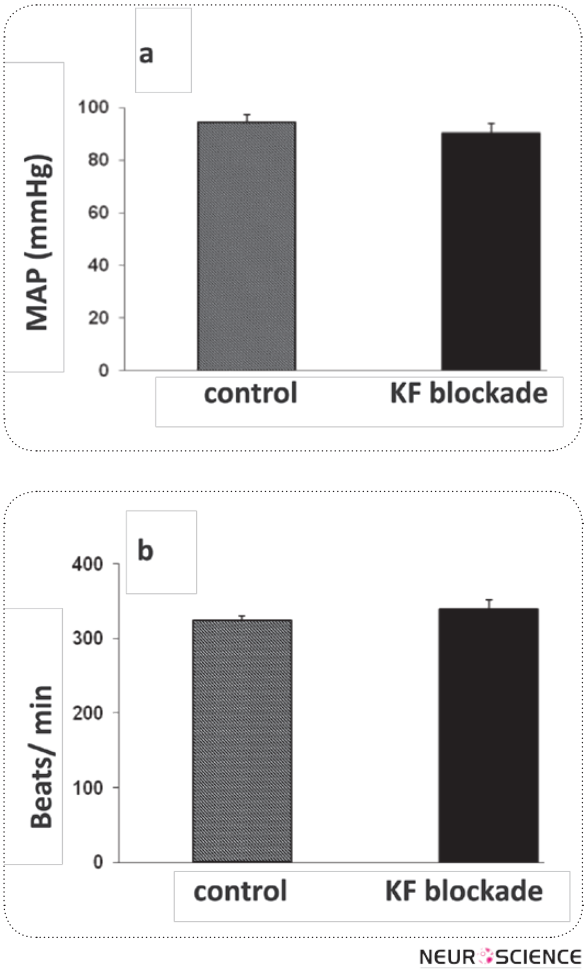

Figure 2. A typical tracing, showing the effects of microinjection of CoCl2 (1mM in100 nl) into the KF nucleus on (a) heart rate and (b) blood pressure. The vertical lines show the time of injection.

4. Discussion

In the present study, we determined the role of KF nucleus on tonic control of cardiovascular system. Our results showed that inactivation of KF nucleus by CoCl2 had no significant effect on basic mean arterial blood pressure and HR. Microinjection of CoCl2 into the brain area is a well known method for evaluating the role of specific areas in the brain in physiological functions. In this method, CoCl2, non-selectively blocks pre-synaptic Ca2+ influx and inhibits the neurotransmitter release without affecting the passage of fibers (Broadbent, Squire, & Clark, 2006; Kretz, 1984; Lomber, 1999). Therefore, it is possible to identify the brain areas involved in information processing and regulation of a specific physiologic function.

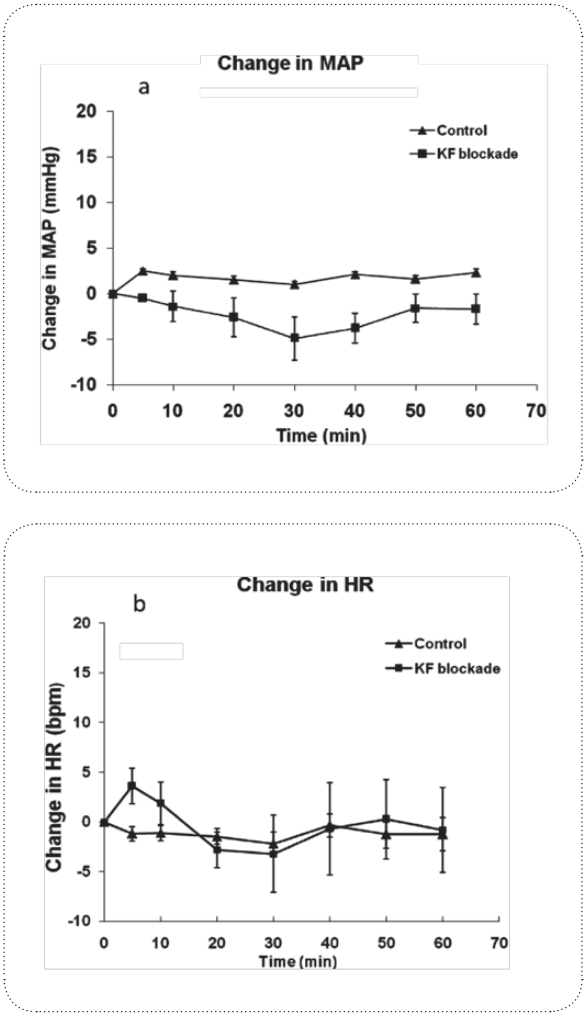

Figure 3. Time courses of mean arterial pressure (MAP)(a) and heart rate (HR) (b) changes in response to the microinjection of CoCl2 into the KF nucleus (n=10) in anesthetized rats. Microinjection of CoCl2 produced no significant effect on MAP and HR.

In our study, blockade of the KF by CoCl2 did not affect the basal MAP and HR. It is suggested that pathways which synapse on the vasomotor neurons in KF nucleus are not involved in maintenance of vasomotor tone and maybe this nucleus has no role in regulation of vasomotor tone and cardiovascular values.

Although inactivation of this nucleus has no effect on baseline blood pressure and heart rate, we cannot rule out the effect of this area on cardiovascular system. The KF nucleus has widespread connections with areas involved in cardiovascular regulation including: RVLM, NTS, CnF, raphe nucleus, PAG and the IML column in spinal cord (Dampney & Horiuchi, 2003; Korte et al., 1992; Shafei & Nasimi, 2011). Involvement of KF nucleus in regulation of cardiovascular responses has been shown in several experiments. Electrical stimulation of the KF nucleus causes pressor and mild tachycardic effect (Korte et al., 1992). In addition, KF nucleus is involved in chemoreceptor reflex (Dampney & Horiuchi, 2003) and relaying of cardiovascular responses of CnF nucleus (Bohus, Koolhaas, & Korte 1996; Korte et al., 1992; Shafei & Nasimi, 2011). Stimulation of cardiac sympathetic afferents also evokes large numbers of KF neurons (Dutschmann & Herbert, 1997; Lam, Gundlach, & Verberne, 1996). Anatomical studies have shown that KF receives inputs from the NTS and has descending projections to the RVLM and the IML of spinal cord (Dampney & Horiuchi, 2003; Guo, Li, & Longhurst, 2002). This, suggests the involvement of KF nucleus in baroreflex activity.

It is reported that several nuclei involved in cardiovascular regulation are subject to the tonic inhibition of GABAergic neurons (Dampney et al., 2003; Mandel & Schreihofer, 2008). It is possible that, same as other nuclei, vasomotor neurons of KF are also strongly inhibited by GABAergic neurons. Therefore, KF nucleus neurons in resting condition have too little activity. However, a stressor factor such as hypoxia, decrease of the arterial pressure, hemorrhage or pain may reduce tonic inhibitory of GABA and activates them.

In addition, previous studies have shown that KF is an integrative area and has several connections with central regulating respiratory areas, autonomic and limbic systems (Bohus et al , 1996; Dampney, 1994; Dampney & Horiuchi, 2003; Guo et al., 2002; Korte et al., 1992). Therefore, this area may be involved in integration of cardiovascular, especially cardio respiratory, inputs. However, further studies are needed to clarify the role of KF nucleus in cardiovascular regulation. In summary, present study indicated that the KF nucleus is not involved in tonic regulation of cardiovascular system but it may be involved in organization of cardio respiratory responses.

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