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RESEARCH ARTICLE

Role of vagus nerve in algogen-induced vasosensory reflex responses in anesthetized rats

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ABSTRACT

Background: It has been shown by various workers that intra-arterial (i.a.) instillation of algogens produced reflex cardiorespiratory (CVR) changes in anesthetized rats, substantiating the role of medium sized peripheral blood vessels in regulation of CVR system. **Aim and Objective**: The aim of this study was to understand the role of vagus nerve in algogen-induced vasosensory reflex responses altering CVR parameters. **Material and Methods:** Femoral artery was cannulated retrogradely and was utilized for the instillation of saline/bradykinin and recording of blood pressure (BP), using a double-ported 24G cannula. BP, respiration and electrocardiogram were recorded for 30 min after instillation of bradykinin with or without vagotomy. Bradykinin (1 μM) was used as an algogen for the elicitation of vasosensory reflex responses altering CVR parameters. **Results:** Instillation (i.a.) of bradykinin produced immediate (5–8 s) hypotensive (40% of initial), bradycardiac (17% of initial), tachypnoeic (45% of initial), and hyperventilatory (96% of initial) responses. In the vagotomized rats, bradykinin-induced hypotensive (18% of initial), bradycardiac (1% of initial), tachypnoeic (5% of initial), and hyperventilatory (10% of initial) responses attenuated significantly. **Conclusion:** Pre-treatment with bilateral vagotomy significantly attenuated the mean arterial pressure, heart rate, respiratory frequency and respiratory minute volume responses indicating the role of vagus in producing these responses.

KEY WORDS: Nociception; Vasosensory Reflexes; Bradykinin; Vanilloid Receptor 1; Cardiorespiratory Changes

INTRODUCTION

Whilst the sensory function of specialized arterial barosensory and chemosensory afferents in regulation of cardiorespiratory (CVR) system has been established;[1-4] that of afferents originating from peripheral blood vessels remains unknown. In the reports elsewhere, it has been suggested that the vasosensory nerves may be involved in reflex regulation of the CVR system.[5,6] Several researchers have demonstrated that intra-arterial (i.a) injection of nociceptive agent produced vasosensory reflex responses altering CVR changes involving various receptors.[7-10] Previously, it has been shown that i.a. injection of algogens produced immediate hypotensive, bradycardiac, tachypnoeic and hyperventilatory changes of shorter latencies.[7-9] Histamine, 5-HT, substance P, calcitonin gene-related peptide, neurokinin-A, and endothelin-3, each of which plays a key role in the pathogenesis of numerous diseases, including migraine, psoriasis, asthma, and eczema, have been implicated in this process.[11] Vanilloid receptor 1 (VR1), a cation channels have been implicated to be located at the sensory nerve terminals arising from the tunica adventitia of the peripheral blood vessels.[5,6] These neurons signal central nervous system about the noxious alterations in their vicinity and hence they are precisely called as perivascular nociceptive afferents.[12,13] Activation of perivascular transient receptor potentials (TRP) channels causes reflex alterations

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in the CVR parameters. These reflex CVR responses are described as vasosensory reflex responses elsewhere.[8,9]

It has been shown that VR1 receptors can be activated by a variety of stimuli such as capsaicin, anandamide, metabolites of poly-unsaturated fatty acids, and noxious heat.[14-16] Recently, it has been shown in our lab that i.a. instillation of an algogen (bradykinin, 1 μM) produced immediate hypotension, bradycardia, tachypnea, and hyperventilation of shorter duration with shorter latencies.[17] Bradykinin was used as a tool for the elicitation of vasosensory reflex responses as it is a potent nociceptive agent forming one of the important components of the inflammatory soup. The role of vagus nerve in mediating these reflex responses is still not clear. Therefore, the present study was designed to investigate the effect of bilateral vagotomy on algogen-induced reflex alterations in mean arterial pressure (MAP), heart rate (HR), respiratory frequency (RF), and respiratory minute volume (RMV) in anesthetized rats.

MATERIALS AND METHODS

Experiments were performed on three groups of rats ($n = 6$). In the first group, saline was injected as a control and the CVR responses were observed for 30 min. In the second group, bradykinin-induced vasosensory reflex responses altering CVR parameters were elicited. In the third group, bradykinin-induced vasosensory reflex response was elicited in the vagotomized animals. Laboratory temperature was maintained at around 28°C ± 2 during the experiments.

Animals and Anesthesia

Ethical clearance was obtained from the Institute Animal Ethical Committee, Banaras Hindu University (Ref. No. Dean/2019/IAEC/1627 dated 17.11.2019) before the commencement of animal experiments. All the animals were handled in accordance with the Indian National Science Academy's "Use of animals in scientific research and education." Animals were housed to 12:12 h light/dark cycle and food/water was provided *ad libitum*. Urethane was freshly prepared in double distilled water in the concentration of 0.5 g/ml. Healthy male albino rats were anesthetized with an intra-peritoneal injection of urethane (1.5 g/kg). A 50–100 mg of anesthesia was given as per the requirement.

Dissection and Recording

Under the effect of anesthesia, tracheostomy was performed to keep the respiratory tract patent by inserting a polyethylene tube. Tracheal secretions were aspirated as and when required. The area of femoral triangle was dissected to isolate the femoral artery from the surrounding structures. A 24G, double-ported polyethylene cannula filled with freshly prepared heparinized saline (20 IU/ml) was inserted into the femoral artery retrogradely. The double port of cannula facilitated simultaneous blood pressure (BP) recording through its horizontal port and instillation of saline/drugs through its vertical port. This method of single cannulation for BP monitoring and drug instillation minimizes the surgical injury to the animal and also marks an instillation artefact by interrupting the BP recording for a fraction of second. Common carotid artery cannulation was avoided as it may compromise the circulation to the pontomedullary areas. The horizontal port of the cannula was further connected to a pressure transducer which in turn was connected to a bridge amplifier and finally to the data acquisition system (Power Lab, AD Instruments, Australia) which provides the MAP on the computer screen.

The respiratory movements were recorded by securing the skin over xiphisternum and connecting it to a force transducer through a thread. The electrocardiographic (ECG) potentials were recorded using needle electrodes, connected in standard limb lead-II configuration. The BP, respiratory excursions, and ECG were recorded on a personal computer. The MAP, HR, RF, and RMV were obtained from the original recordings of the experiments. At the end of experiments, animals were sacrificed by overdose of anesthesia. The deflection of chest wall was then recorded by introducing a known volume (1 ml) of air into the quiescent lung through the tracheal tube and was computed as "x." For calculation of ventilation, the average amplitude (h) of respiratory excursions was measured. The height (mm) of respiration was then converted to volume (ml) using the calibration (h/x). RMV was then calculated by multiplying the RF with (h/x).

Drugs and Solutions

Urethane was procured from Merck, Germany and was freshly prepared in double distilled water (0.5 g/ml) before each experiment. Bradykinin acetate salt was procured from the Sigma Chemicals Company, St Louis, USA. Stock solution of bradykinin (1 mg/ml) was prepared in double distilled water and refrigerated. Subsequent dilutions were made in normal saline at the time of experimentation. Volume of all the drugs was kept minimum and constant (100 μl) to minimize the stretch-induced responses and systemic spillage. Heparin (1000 U/ml) was obtained from Biological Evans Ltd., Hyderabad, India and heparinized saline (20 IU/ml) was used to fill the transducer and cannula.

Experimental Protocol

Determination of bradykinin-induced cardiorespiratory responses

After dissection, 15 min were given to the animals for the stabilization of CVR parameters. Then, the initial recordings of respiration, BP, and ECG were performed. Normal saline (100 μl) was instilled intra-arterially and CVR parameters were recorded for 15 min. Further, bradykinin was instilled

into the same femoral artery and the recordings of the CVR parameters were made for 30 min. The normal saline recordings served as time-matched control responses.

Effect of bilateral vagotomy on bradykinin-induced cardiorespiratory changes

In a separate group of animals, the initial recordings of respiration, BP, and ECG were performed as mentioned earlier. Bilateral vagotomy was performed and the CVR parameters were recorded for 15 min to see the effect of vagotomy on CVR parameters. This was followed by the instillation of bradykinin into the same femoral artery and the CVR parameters were recorded for 30 min as mentioned in earlier paragraph.

Analysis of Data and Statistics

The data were presented as % of initial values at various time intervals. The values at a given time were pooled and were presented as mean ± SEM. The statistical significance between two groups was analyzed by comparing the MAP, HR, RF, and RMV responses of bradykinin only group and bradykinin after vagotomy group. The comparisons of various groups were made using *post-hoc* correction, Dunnett's test (two sided), and for the comparisons with their initial values using Student's paired *t*-test, using SPSS-16.0 software. $P < 0.05$ was considered statistically significant.

RESULTS

Instillation of bradykinin into the femoral artery produced immediate hypotensive, bradycardiac, tachypnoeic, and hyperventilatory responses within 5–8 s. Since, 1 μM of bradykinin produced optimal responses on all cardiorespiratory (CVR) parameters; therefore, this concentration was used as a tool for the elicitation of vasosensory reflex responses.[17] The MAP, HR, RF, and RMV responses before agonist were considered as initial responses. CVR changes were observed for 30 min but it was found that no significant changes took place after 2 min.[17]

Effects on MAP before and after Bilateral Vagotomy

In the stabilized animals, the resting MAP was 80.3 ± 3.92 mm Hg. Instillation of saline did not produce any changes in the MAP up to 30 min. Instillation of bradykinin elicited immediate hypotensive changes (40% of initial) which was significantly different than the time-matched saline group [Table 1; Figures 1 and 2; $P < 0.05$, *post-hoc* correction using Dunnett's test (two sided)] or the corresponding initial value [Figures 1 and 2; $P < 0.05$, Student's *t*-test for paired observations]. The CVR changes returned back to the initial level within 2–4 min of instillation of bradykinin and remained at that level for 30 min [Figures 1 and 2].

After bilateral vagotomy (B/L vagx), there was no significant change in the resting MAP (84.3 ± 2.28 mm Hg) after 15 min. Instillation (i.a.) of saline did not produce any changes in the MAP up to 15 min. In this group, the bradykinin-induced hypotensive changes (18% of initial) were attenuated markedly which were significantly different than the time-matched bradykinin only responses [Table 1 and Figures 1 and 2; $P < 0.05$, *post-hoc* correction using Dunnett's test (two sided)] or the corresponding initial value [Figures 1 and 2; $P < 0.05$, Student's *t*-test for paired observations].

Effects on HR before and after Bilateral Vagotomy

After stabilization of the animals, resting HR was 322.8 ± 7.20 beats per min. Instillation (i.a.) of saline did not produce any changes in the HR up to entire observation period. Instillation of bradykinin produced immediate bradycardiac changes (17% of initial) which was significantly different than the time-matched saline group [Table 1; Figures 1 and 2; $P < 0.05$, *post-hoc* correction using Dunnett's test (two sided)] or the corresponding initial value [Figures 1 and 2; $P < 0.05$, Student's *t*-test for paired observations].

Table 1: Effect of saline/bilateral vagotomy on bradykinin-induced cardiorespiratory changes. The data are presented as mean±SEM from six different experiments. The "Before" values indicate the values 10 s before instillation of drug and the "After" values 10 s after the drug

Agonist/intervention	MAP (mm Hg)	HR (beats/min)	RF (per min)	RMV (ml/min)
Saline				
Before	83.14±1.45	336.0±6.53	84.5±2.87	167.0±4.90
After	82.42±0.82	334.7±5.90	87.2±2.96	189.5±7.10
Bradykinin only (1 μM)				
Before	82.0±4.15	321.2±4.22	86.7±2.67	152.3±8.40
After	49.0±3.67	266.7±4.22	125.3±5.63	298.5±12.01
+ Bilateral vagotomy				
Before	84.7±2.67	301.0±1.63	39.7±1.52	109.6±4.54
After	69.3±4.14	298.2±1.19	41.8±1.68	120.5±5.09

MAP: Mean arterial pressure, HR: Heart rate, RF: Respiratory frequency, RMV: Respiratory minute volume, BK: Bradykinin

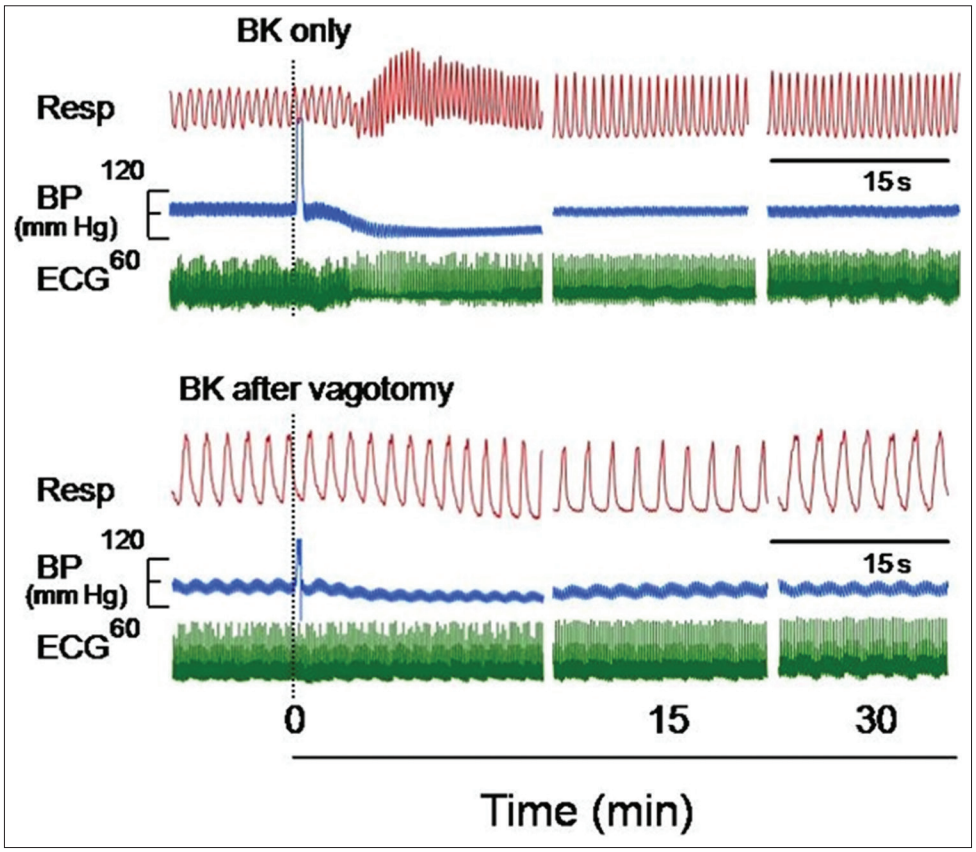

Figure 1: Original recordings at different time intervals of an experiment are shown to depict the effect of intra-arterial instillation of bradykinin (BK) in naive animals or after bilateral vagotomy on respiration (Resp), blood pressure (BP), and electrocardiogram (ECG). The time-scale is shown in the lower panel. Dotted lines indicate the point of instillation of drug

After B/L vagotomy, there was no significant change in the resting HR (300.2 ± 1.85 mm Hg). In this group, the bradykinin-induced bradycardiac changes (1.5% of initial) were blocked significantly than the time-matched bradykinin only responses [Table 1, Figures 1 and 2; $P < 0.05$, *post-hoc* correction using Dunnett's test (two sided)] or the corresponding initial value [Figures 1 and 2; $P < 0.05$, Student's *t*-test for paired observations].

Effects on RF before and after Bilateral Vagotomy

After dissection, the resting RF was 83.0 ± 2.93 per min. Instillation (i.a.) of saline did not produce any changes in the RF up to 30 min. Instillation of bradykinin produced immediate tachypnea (45% of initial) which was significantly different than the time-matched saline group [Table 1; Figures 1 and 2; $P < 0.05$, *post-hoc* correction using Dunnett's test (two sided)] or the corresponding initial value [Figures 1 and 2; $P < 0.05$, Student's *t*-test for paired observations].

In this group of animals, initial RF was 78.3 ± 1.89. After B/L vagotomy, there was drastic decrease in the resting RF (40.3 ± 1.58). Bradykinin-induced tachypnoeic responses (5% of initial) were blocked significantly than the time-matched bradykinin responses [Table 1, Figures 1 and 2; $P < 0.05$, *post-hoc* correction using Dunnett's test (two sided)] or the corresponding initial value [Figures 1 and 2; $P < 0.05$, Student's *t*-test for paired observations].

Effects on RMV before and after Bilateral Vagotomy

After dissection, the resting RMV was (RMV) 153.0 ± 8.40 ml/min. Instillation of saline did not produce any changes in the RMV up to 30 min. Instillation of bradykinin produced immediate hyperventilation (96 % of initial) which was significantly different than the time-matched saline group [Table 1; Figures 1 and 2; $P < 0.05$, *post-hoc* correction using Dunnett's test (two sided)] or the corresponding initial value [Figures 1 and 2; $P < 0.05$, Student's *t*-test for paired observations].

In these animals, initial RMV was 147.7 ± 5.75. After B/L vagotomy, there was drastic decrease in the resting RMV (111.7 ± 6.24). Bradykinin-induced hyperventilatory responses (10% of initial) were blocked significantly than the time-matched bradykinin responses [Table 1 and Figures 1 and 2; $P < 0.05$, *post-hoc* correction using Dunnett's test (two sided)] or the corresponding initial value [Figures 1 and 2; $P < 0.05$, Student's *t*-test for paired observations].

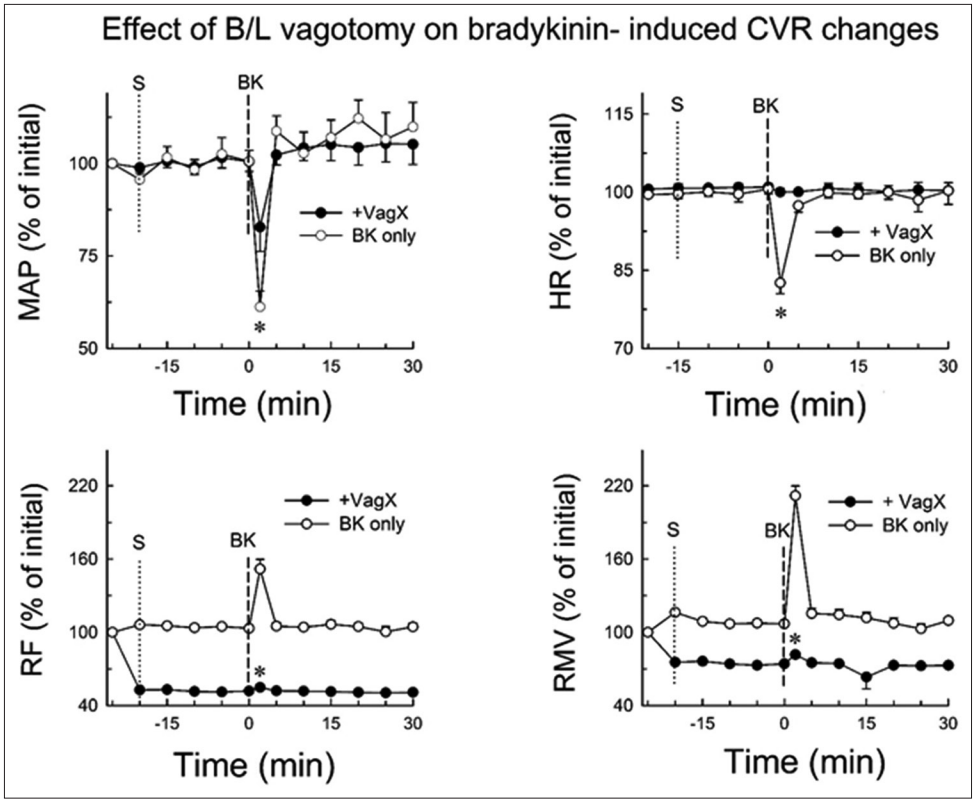

Figure 2: Time-response relationships of bradykinin (BK) on mean arterial pressure (MAP), heart rate (HR), respiratory frequency (RF), and respiratory minute volume (RMV). Pre-treatment with bilateral vagotomy, blocked the bradykinin-induced HR changes and respiratory changes, and significantly attenuated the MAP changes. The mean ± SEM values from six experiments in each group are presented in line graph. The MAP, HR, RF, and RMV responses are significantly attenuated in vagotomy group as compared to the BK only group ($P < 0.05$, *post-hoc* correction using Dunnett's *t*-test [two sided]). Dotted lines indicate the point of injection of saline/BK (1 μM). An asterisk **(*)** indicates significant difference of responses as compared to BK only group. B/L = Bilateral, BK = bradykinin, S = Saline, CVR = Cardiorespiratory

DISCUSSION

Observations of this study demonstrate that instillation of bradykinin into a segment of femoral artery produces immediate hypotensive, bradycardiac, tachypnoeic, and hyperventilatory changes of shorter latencies, while equivolume of normal saline did not produce any CVR responses, excluding the possibility of stretch/ischemia-induced responses on the vessel wall. After bilateral vagotomy, bradykinin-induced vasosensory reflex responses were significantly attenuated leading to decreased MAP, HR, RF, and RMV responses. All the responses were short-lived and all the parameters returned back to the initial level within 2 min from the point of instillation of the agonist.

In the past, various researchers have shown that the intravascular injection of different chemical nociceptive agonists such as capsaicin, ATP, anandamide,[8] acrolein,[18] and bradykinin[19] produced reflex CVR changes. Earlier works from this laboratory have also demonstrated the reflex responses elicited by activation of perivascular afferent neurons using Indian red scorpion venom as a nociceptive agent.[20-23] In a study elsewhere using single-unit extracellular recording techniques, the role of the VR1 aka TRPV1 in bradykinin-induced activation of vagal afferent C-fiber receptive fields in guinea pig isolated airways has also been demonstrated.[24] In the present study, we observed that the bradykinin-induced CVR changes were significantly attenuated in the vagotomized rats. The possible reasons behind the attenuation of CVR responses are that the vagus carries several afferent and efferent fibers from the heart and lung. It is well known that parasympathetic fibers modulating HR run through the vagus which directly modulates the HR.[23] The afferent fibers which carry the information regarding the status of inflation of lungs also run through the vagus. Hence, after bilateral vagotomy the rate of respiration becomes sluggish and deep. It is clearly demonstrated in Figure 2 that after vagotomy, the resting level of RF and RMV is different than the control group. Although, the increase in depth of respiration was observed after vagotomy but could not compensate the minute ventilation completely. As for as the attenuation in MAP responses are concern, these responses are least attenuated in comparison to the other CVR parameters probably because of the indirect involvement of the sympathetic nervous system in producing these responses. Our observations are also consistent with the similar findings found elsewhere.[23]

In this study, a novel method of experimental design was used for the instillation of nociceptive agent by retrograde

single cannulation of femoral artery. The cannula was soldered with the double-ported inlet which was used to record the BP and to inject the agonist simultaneously.[17] This reduced the surgical injury to the animal and also marked an artefact which accurately denoted the point of instillation of saline/drugs. However, the exact anatomical location of the perivascular afferents could not be identified in this study and the possibility of involvement of other sensory afferents cannot be ruled out completely.

CONCLUSION

Thus, it can be concluded that the bradykinin-induced vasosensory reflex responses modulating CVR parameters, that is, MAP, HR, RF, and RMV, involve vagus at least for the regulation of HR and pulmonary ventilation directly and MAP regulation, indirectly.

REFERENCES

1. Heymans C, Neil E. Reflexogenic areas of the cardiovascular system. BJS Soc 1958;46:92.
2. Shepherd JT. The lungs as receptor sites for cardiovascular regulation. Circulation 1981;63:1-10.
3. Marshall JN. Peripheral chemoreceptors and cardiovascular regulations. Physiol Rev 1994;74:543-94.
4. Hainsworth R. Cardiovascular reflexes from ventricular and coronary receptors. Adv Exp Med Biol 1995;381:157-74.
5. Donnerer J, Lembeck F. Analysis of the effects of intravenously injected capsaicin in the rat. Naunyn Schmiedebergs Arch Pharmacol 1982;320:54-7.
6. Donnerer J, Lembeck F. Capsaicin-induced reflex fall in rat blood pressure is mediated by afferent substance P-containing neurons via a reflex centre in the brainstem. Naunyn Schmiedebergs Arch Pharmacol 1983;324:293-5.
7. McQueen DS, Bond SM, Moores C, Chessell L, Humphrey PP, Dowd E. Activation of P2X receptors for adenosine triphosphate evokes cardiorespiratory reflexes in anaesthetized rats. J Physiol 1998;359:1-18.
8. Smith PJW, McQueen DS. Anandamide induces cardiovascular and respiratory reflexes via vasosensory nerves in anesthetized rat. Br J Pharmacol 2001;134:655-63.
9. Singh SK, Deshpande SB. Intra-arterial injection of *Mesobuthus tamulus* venom elicits cardiorespiratory reflexes involving perivascular afferents. Toxicon 2005;46:820-6.
10. Singh SK, Deshpande SB. N-methyl-D-aspartate receptors mediate *Mesobuthus tumulus* venom-induced vasosensory reflex responses in anesthetized rats. Natl J Physiol Pharm Pharmacol 2017;7:679-84.
11. Rosa AC, Fantozzi R. The role of histamine in neurogenic inflammation. Br J Pharm 2013;170:38-45.
12. Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature 2001;413:203-10.
13. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: A heat-activated ion channel in the pain pathway. Nature 1997;389:816-24.
14. Mickle AD, Shepherd AJ, Mohapatra PD. Sensory TRP channels: The key transducers of nociception and pain. Prog Mol Biol Transl Sci 2015;131:73-118.
15. Raja SN, Meyer RA, Campbell JN. Peripheral mechanisms of somatic pain. Anaesthesiology 1988;68:571-90.
16. Hees JV, Gybels J. Nociceptor activity in human nerve during painful and non-painful skin stimulation. J Neurol Neurosurg Psychiatry 1981;44:600-7.
17. Singh SK, Mandal MB, Revand R. Instillation of bradykinin in femoral artery elicits cardiorespiratory reflexes involving perivascular afferents in anesthetized rats. Physiol Int 2020;107:40-5.
18. Tsagareli MG, Tsiklauri N, Zanotto KL. Behavioral evidence of thermal hyperalgesia and mechanical allodynia induced by intradermal cinnamaldehyde in rats. Neurosci Lett 2010;473:233-6.
19. Smith PJW, McQueen DS. Perivascular nerves induce cardiorespiratory reflexes in response to algogens in anesthetized rats. Neurosci Res 2004;50:271-81.
20. Singh SK, Deshpande SB. Injection of *Mesobuthus tamulus* venom in distal segment of femoral artery evokes hyperventilatory and hypertensive responses in anaesthetised rats. Neurosci Lett 2008a;438:64-6.
21. Singh SK, Deshpande SB. Vasosensory responses elicited by Indian red scorpion venom last longer than capsaicin-induced responses. Indian J Exp Biol 2008;46:755-9.
22. Singh SK, Deshpande SB. Nociceptive vascular reflexes evoked by scorpion venom modulates cardiorespiratory parameters involving vanilloid receptor 1. Neurosci Lett 2009;451:194-8.
23. Singh SK, Deshpande SB. *Buthus tamulus* venom-induced vasosensory reflexes are mediated through efferent pathways in sympathetic and vagal parasympathetics. Neurosci Lett 2009;464:199-202.
24. Carr MJ, Kollarik M, Meeker SN, Undem BJ. A Role for TRPV1 in bradykinin-induced excitation of vagal airway afferent nerve terminals. J Pharmacol Exp Ther 2003;304:1275-9.

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