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RESEARCH ARTICLE

N-methyl-D-aspartate receptors mediate *Mesobuthus tamulus* venom-induced vasosensory reflex responses in anesthetized rats

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ABSTRACT

Background: The autonomic changes and cardiorespiratory changes seen in vascular disorders are suggested to be mediated reflexly by the activation of perivascular nociceptors. The role of prostaglandins, vanilloid receptor 1, 5-hydroxytryptamine type 3, and kinin receptors is well established. **Aims and Objectives:** This study was conducted to understand the role of N-methyl-D-aspartate (NMDA) receptor in modulating vasosensory reflex responses evoked by *Mesobuthus tumulus* venom. **Materials and Methods:** Healthy male albino rats were anesthetized with an intra-peritoneal injection of urethane (1.5 g/kg). Tracheostomy was performed to keep the airway patent. Femoral artery was cannulated proximally as well as distally to record the blood pressure (BP) and to inject the chemicals, respectively. The effect of venom on BP, heart rate (HR), respiratory rate, and minute ventilation was recorded for 60 min at every 5 min and presented as mean ± standard error of mean. **Results:** Intra-arterial injection of venom produced immediate hyperventilatory response (increase of 41.6%), followed by hypoventilatory response (decrease of 40.1%), and finally sustained hyperventilatory response (increase of 53%) which was observed up to 60 min. The hypertensive response started at 40 s, peaking at 5 min (increase of 48%), and remained above the initial level subsequently. The bradycardiac response began around 5 min, peaking at 25 min (decrease of 50% in HR), and remained at that level up to 60 min. In 2-amino 5-phosphonovaleric acid (NMDA receptor antagonist) pre-treated group, the venom-induced cardiovascular responses (mean arterial pressure and HR) were markedly attenuated in comparisons to the venom only group but not the respiratory responses. **Conclusions:** The data provide evidence for the involvement of NMDA receptors in producing the venom-induced vasosensory reflex responses modulating the cardiovascular parameters in anesthetized rats.

KEY WORDS: N-methyl-D-aspartate Receptor; 2-amino 5-phosphonovaleric Acid; Nociception; Vasosensory Reflexes; Cardiorespiratory Changes

INTRODUCTION

Perivascular nociceptors have been implicated in the pain associated with angina, myocardial infarction, migraine, and intermittent claudication.[1,2] The role of large blood vessels in the regulation of cardiorespiratory parameters is well established, but the role of medium-sized peripheral blood vessel in this regard is not known yet. Since the peripheral blood vessels are also of same mesodermal origin, therefore, it is expected that these peripheral blood vessels may contain those receptors which are present in the large blood vessels regulating blood pressure (BP). Peripheral vascular disorders are also implicated in the long-term cardiovascular alterations and other behavioral changes[3,4] favoring our hypothesis.

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It has been shown that intra-arterial (i.a.) injection of capsaicin/αβ methylene adenosine 5′-triphosphate

produces immediate hyperventilatory and hypotensive responses,[5,6] indicating the role of peripheral blood vessels in the modulation of cardiorespiratory system. However, these observations were limited for very short period (10 s) in contrast to the long lasting autonomic changes seen in the chronic vascular diseases. It has been shown that i.a. injection of *Mesobuthus tumulus* (BT) venom elicits reflex cardiorespiratory changes lasting for more 30 min.[7-9] These changes were categorized as immediate-tachypneic, intermediate-hypertensive, and delayed-bradycardiac responses.[7] Therefore, in this study, BT venom (1 mg/kg) was used to stimulate the perivascular nociceptors. Further, the responses are shown to be mediated by prostaglandins (PGs) at the peripheral end[7] and the afferents for these reflex responses are mediated mainly through the ipsilateral somatic nerve.[8] The venom-induced vasosensory reflex responses lasts longer than the other nociceptive agonists (capsaicin/anandamide/ αβMe-ATP)-induced responses.[6,9,10] It was also shown that nociceptive vascular reflex responses evoked by BT venom modulate cardiorespiratory parameters involving transient receptor potential vanilloid 1 (TRPV1)[11] and the efferents are located in the sympathetic and vagal parasympathetics.[12] Recently, the role of B1-kinin receptors has also been shown in the venom-induced vasosensory reflex responses.[13]

The cardiorespiratory alterations seen in autonomic changes in vascular disorders are suggested to be mediated reflexly by the activation of sensors around the peripheral blood vessels.[3,4] The role of TRPV1, 5-hydroxytryptamine type 3 (5-HT_3) receptor, and B1-kinin receptor is well established in the venom-induced vasosensory reflex responses.[11,13] Since the 2-amino 5-phosphonovaleric acid (APV) is implicated in the modulation of different kind of pain in the animal model, therefore, the present study was conducted to examine the effect of APV in mediating the venom-induced vasosensory reflex responses.

MATERIALS AND METHODS

Animals and Anesthesia

All the experiments were performed in the Department of Physiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, as per the guideline given by the Institute Ethical Committee (approval number-Dean/13-14/ CAE/189). Experiments were performed on healthy albino rats (Charles-Foster strain) weighing between 200 and 300 g. Animals were anesthetized with urethane (Merck, Germany), with an initial dose of 1.5 g/kg body weight, intra-peritoneally and was maintained by injecting the urethane as required. The animals were exposed to the 12:12 h light/dark cycle to keep diurnal variations intact and were provided with *ad libitum* animal food (Hindustan Lever Ltd.) and water.

Dissection and Recordings

The tracheal cannulation was done to keep the respiratory way patent. Trachea was exposed by making a mid-line incision over the neck. A transverse cut was made between the tracheal rings, and a polyethylene tube of appropriate diameter was inserted and secured firmly by a thread. Tracheal secretions were aspirated by gentle suction through a fine polyethylene tube. Femoral triangle was exposed by making an incision along the course of femoral artery. The femoral artery was dissected by clearing the tissues and fascia from the surrounding structures. The femoral vein and femoral nerve were separated out from the artery. Freshly prepared heparinized saline (20 IU/ml) was loaded in the syringe attached with appropriate-sized cannula. A small nick was made in the femoral artery proximally and the cannula was inserted and secured firmly with thread. Later on, the cannula was connected through a tri-way stop cock to the Statham strain gage pressure transducer (Biodevices, Ambala). After cannulation of proximal segment of the femoral artery, the distal segment of the same artery was cannulated to inject the drugs/venom/saline in a local segment of the vessel. The placement of cannula was confirmed by injecting 0.10 ml saline.

The pressure transducer was filled with the heparinized saline and connected with tri-way stop cock, which in turn was connected with artery through cannula. The free pulsatile flow of blood was obtained after releasing the thread clamp. The pressure transducer was connected to a bridge amplifier through a galvanometer. The galvanometer deflections were recorded on a chart paper with the help of a writing pen. The mean arterial pressure (MAP) was computed from the recording and was considered as the parameter for BP throughout the study. The instrument was calibrated in between the experiments as per the need. The needle electrodes were connected as per the standard limb lead II configuration for the recording of electrocardiogram (ECG). The electrocardiographic potentials were recorded on a chart recorder. Heart rate (HR) was computed manually from R-R intervals of the ECG. The force displacement transducer was connected to the chest with the thread by securing skin over xiphisternum. The respiratory movements were recorded on a chart recorder through a bridge amplifier. Respiratory rate (RR) and minute ventilation (MV) were computed from the respiratory excursions.

After the dissection, 30 min was given for the stabilization of the vital parameters which was followed by the initial recording of BP, ECG, and respiratory movements. Then, 0.10 ml of normal saline was injected in the peripheral segment of femoral artery, and the cardiorespiratory parameters were recorded at every 5 min up to 20 min as initial recording. This was followed by the injection of venom (1.0 mg/kg) in the peripheral segment of the same femoral artery, and the cardiorespiratory parameters were recorded at every 5 min up to 60 min.

Drugs and Solutions

Crude BT venom was purchased from the Haffkine Institute, Mumbai, India. 2 mg/ml stock solution of BT venom was prepared in the distilled water and was refrigerated. 1 mg/kg BT venom was freshly prepared from the stock solution and injected to stimulate the perivascular nociceptors as it produces optimal responses on the cardiorespiratory parameters. Heparin was obtained from Biological Evans Ltd., Hyderabad, India. APV (N-methyl-D-aspartate [NMDA] receptor antagonist) was obtained from Sigma Chemical Company, St. Louis, MO, USA.

Experimental Protocol

Twelve animals were used in this study in two different groups ($n = 6$ for each group). All the chemicals/venoms/saline was injected in the peripheral segment of femoral artery as the proximal segment was connected with the Statham strain gage pressure transducer for the BP recording. In the first group, BT venom was administered i.a. and the BP, ECG, and respiratory movements were recorded. The MAP, HR, RR, and MV were computed manually at every 5 min up to 60 min. These responses were considered as the "venom-only" responses. In the second group, APV (NMDA receptor antagonist) was injected and the cardiorespiratory parameters were recorded for 10 min. Subsequently, BT venom was injected in the APV pre-treated group. The BP, ECG, and respiratory movements were recorded and the MAP, HR, RR, and MV were computed at every 5 min up to 60 min. Volume of the injectables was kept constant (0.10 ml) to avoid the effect of stretch on the vessel wall, and the room temperature was maintained at ~ 25°C throughout the experiment.

Analysis of Data and Statistics

The results were presented as mean ± standard error of mean (SEM) values. The MAP, HR, RR, and MV responses before venom were considered as initial responses. The comparisons of both groups were done using the two-way analysis of variance (ANOVA) test. Student's *t*-test was also done wherever required. $P < 0.05$ was considered statistically significant.

RESULTS

In this study, BT venom (1 mg/kg) was administered to stimulate the perivascular nociceptors. Venom was injected in the peripheral segment of femoral artery to stimulate the perivascular nociceptors as this concentration produced optimal responses on the cardiorespiratory parameters in the anesthetized rats.[7] Therefore, this concentration was used as a tool for the elicitation of vasosensory reflex responses in this study. In the first group, six ($n = 6$) rats were used to examine the effect of BT venom on the cardiorespiratory parameters. In the second group, six ($n = 6$) rats were pre-treated with APV (40 μg/kg) and the venom-induced vasosensory responses were recorded. Individual and mean ± SEM values of MAP, HR, RR, and MV are given after the injection of venom-only and after the injection of venom in APV pre-treated group (Fig 1 and 2). The original tracings are also shown in the Fig 1.

Effects of APV (NMDA Receptor-antagonist) on Venom-induced Cardiorespiratory Alterations

After administration of venom in control group (venom only), there was immediate decrease in MAP (from 90 ± 4.9 to 87 ± 6.7 mmHg) followed by an increase which reached the peak level (131 ± 6.8 mmHg) within 5 min. Later on, MAP decreased but still remained above the initial level up to 60 min (Fig 1 and 2). After administration of APV, there was no change in resting MAP level. In APV pre-treated group, venom did not produce any change in the immediate depressor response, but the hypertensive response was attenuated markedly up to 60 min (Fig 1 and 2). The changes were significantly different from the venom only group during the entire period of observation ($P < 0.05$, two-way ANOVA).

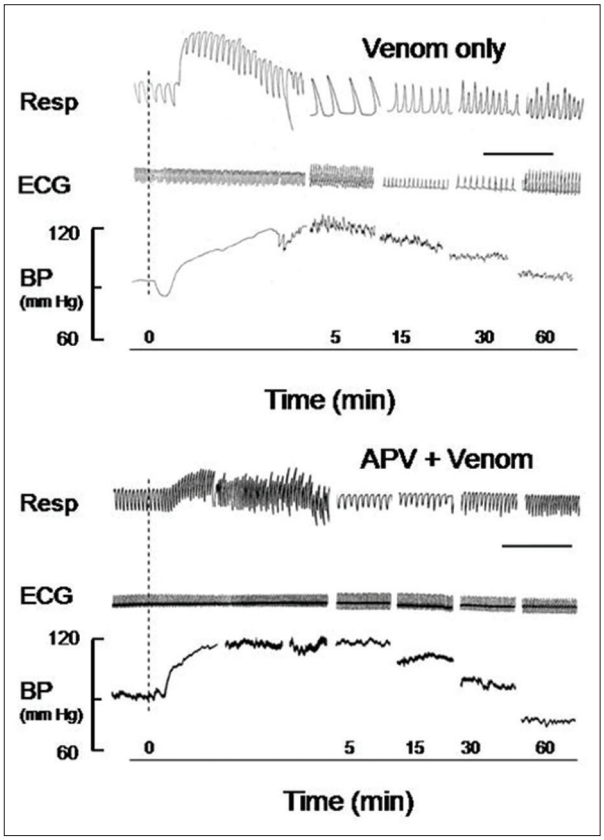

Figure 1: Actual recordings showing the effects of intra-arterial injection of *Mesobuthus tamulus* venom on respiration, electrocardiogram (ECG), and blood pressure (BP). The venom-induced responses are shown at different time intervals as indicated in the lower panel. The point of injection is indicated by dotted line. The unit for calibration of BP is in mmHg. The horizontal line indicates 5 s for respiration and ECG and 50 s for BP

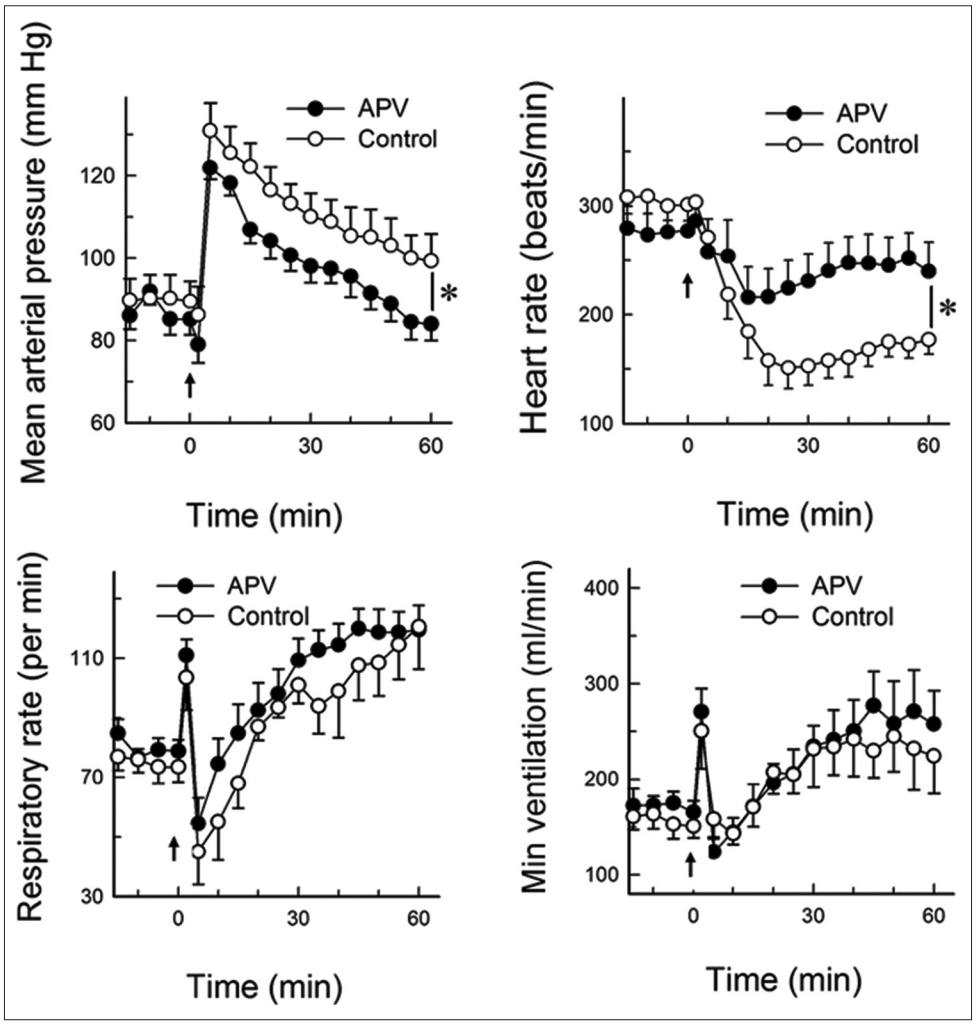

Figure 2: 2-amino 5-phosphonovaleric acid (APV) - 40 μg/kg pretreatment attenuated mean arterial pressure and heart rate changes induced by intra-arterial injection of venom (1 mg/kg). The mean ± standard error of mean values from six experiments is shown in line graphs. Arrows indicate the point of administration of venom. An asterisk (*) indicates $P < 0.05$ (two-way ANOVA) as compared to "venom-only" group from APV pre-treated group (APV + venom)

There was no immediate change in the HR after administration of venom in the control group, but the HR began to decrease within 5 min and the decrease was observed maximal at 25 min (151 ± 18.9 beats/min), which was maintained at that level up to 60 min (Fig 1 and 2). After administration of APV, HR did not change from the initial level. In APV pre-treated group, the bradycardiac response induced by venom was attenuated significantly ($P < 0.05$, two-way ANOVA).

There was an increase in the rate and depth of respiration immediately after the administration of venom in the control group (from 74 ± 5.1 to 104 ± 10.8/min), which was about 40% of the initial level. Then, a decrease in RR was observed below the initial level within 5 min (45 ± 10.9/min). Later on, there was gradual and sustained increase in the RR up to 60 min (Fig 1 and 2). After administration of APV, there was no change in the resting RR. In APV pre-treated group, the venom-induced RR changes were similar to that of control group up to 60 min (Fig 1 and 2). The changes in the MV were similar to the RR changes both in venom-only group and APV pre-treated group (Fig 1 and 2).

DISCUSSION

In our earlier reports, BT venom-induced vasosensory reflex responses altering cardiorespiratory parameters have been demonstrated.[7] Further, it was shown that the afferents are present in the ipsilateral somatic nerves and in nerve plexuses around the blood vessels[8] and the efferents are located in the sympathetic and vagal parasympathetics.[12] It is well known that inflammatory mediators are involved in nociceptive processing at the perivascular nociceptors level. These mediators in turn modulate the VR1, 5-HT_3, and B1 kinin receptors in producing the venom-induced vasosensory reflex responses.[13] All the phases and the parameters of the reflex responses are blocked by the PG synthesis inhibitor.[7]

BT venom contains serotonin, histamine, bradykinin potentiating factor, peptide toxins, etc., which are powerful nociceptive agents.[14-16] Thus, i.a. injection of venom stimulates vast population of nociceptors located around the peripheral blood vessels.

The peripheral sensitization by PGE_2 is mediated through the G-protein coupled receptors that increase cyclic adenosine monophosphate (C-AMP) levels within the nociceptors.[17] The PGs are also involved in nociception at spinal cord synapses. Thus, the PGs are involved at both peripheral and central sites. PGE2 mediated mechanism in dorsal root ganglion is shown to be mediated through voltage-dependent tetrodotoxin-resistant Na^+ channel. This also involves phosphorylation of the channel protein by C-AMP-dependent protein kinase A.[18-20]

5-HT_3 receptor is a pentameric ligand-gated ion channel. It promotes the entry of cations to produce depolarization. Structural features are similar to nicotinic acetylcholine receptors.[21] Two binding sites have been identified, viz., the agonist binding site and antagonist binding site. The agonist binding site in addition to agonist has ethanol recognition/ binding site, while the antagonist binding site, in addition to antagonist, has ketamine recognition/binding site also.[22] The ketamine has a binding site at NMDA receptor also. Thus, the reports provide evidence for the linkage between NMDA and 5-HT_3 receptors.

In addition to the NMDA binding site on 5-HT_3 receptors, the glutamate at NMDA receptors is involved in the nociceptive responses in inflamed regions. The NMDA involvement was further substantiated by its blockade in modulating the visceral nociception.[23,24] In addition, the peripheral terminals of primary afferent nerves innervating somatic tissues also express NMDA receptors.[25-27] Peripheral injection of NMDA receptor antagonist attenuates pain associated with neuropathic or inflammatory origin.[28-32] Thus, the involvements of NMDA receptors are expected in the peripheral processing of nociception.

In the present study, cardiovascular responses (MAP and HR) are primarily antagonized by APV, but there is no any change in the respiratory responses. This finding is consistent with the venom-induced cardiovascular changes seen in ondansetron pre-treated group.[11] Since in this study, only cardiovascular responses are blocked in APV pre-treated group, it is presumable that NMDA receptor blocker interacts with ketamine recognition site of 5-HT_3 receptor.

CONCLUSION

The present results demonstrate the involvement of extracellular inflammatory mediators (bradykinin and PGs) in producing nociceptive vasosensory reflexes modulating the 5-HT_3 receptors and the NMDA receptors. Since 5-HT_3 receptor shares a domain with NMDA receptor, involvement of NMDA receptor is expected in producing the venom-induced vasosensory reflex responses, which is shown in the present result (Fig 1 and 2).

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