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SCORPION (*Buthus tamulus*) VENOM TOXICITY ON CARDIOPULMONARY REFLEXES INVOLVES KININS VIA 5-HT$_3$ RECEPTOR SUBTYPES

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ABSTRACT: The mechanisms underlying the action of Indian red scorpion *Buthus tamulus* (BT) venom-induced augmentation of cardiopulmonary reflexes elicited by intravenous injection of 5-HT were examined in urethane anaesthetized rats. The 5-HT produced a concentration-dependent increase in time-response area of bradycardiac response, with the responses at submaximal concentrations shifted to the left after exposure to BT venom (20 µg/kg, IV). Aprotinin (6000 kallikrein inactivating unit, IV) as such had no effect on 5-HT reflex responses (bradycardia, hypotension, and apnea), but blocked the venom-induced reflex augmentation. While ondansetron (10 µg/kg, IV) completely blocked the 5-HT reflex responses, these reappeared partially after venom exposure (20 µg/kg). Exposure to bradykinin (50 µg/kg, IV) for 30 min also augmented the 5-HT-induced reflex responses similar to venom. The bradykinin-induced augmentation was also blocked by ondansetron. Results indicate that the venom-induced augmentation of cardiopulmonary reflexes is mediated through kinins sensitizing 5-HT$_3$ receptor subtypes.

KEY WORDS: Bezold-Jarisch reflex, aprotinin, bradykinin, indian red scorpion, ondansetron, *Buthus tamulus*, kinins, 5-HT$_3$ receptors, cardiopulmonary reflexes.

INTRODUCTION

Indian red scorpion venom produces fatal toxicity in man and experimental animals. Toxicity was attributed to the cardiovascular abnormalities, such as myocarditis, circulatory failure, autonomic storm, and other metabolic alterations (12,13,14,20). The treatment protocol includes atropine, propranolol, insulin, etc (3,15). Antivenoms have also been tried with some success (8,15). Treatment, however, still remains symptomatic due to the low level of understanding of the pathophysiological changes after envenomation. Reports elsewhere indicate that kinin synthesis inhibitor (aprotinin) prolonged the survival time of rats against *Leiurus quinquestriatus* toxicity and was proposed for treatment of scorpion envenomation (8). Aprotinin also prevented the augmentation of cardiopulmonary reflexes produced by *Buthus tamulus* (BT) venom (2). Further, captopril, an agent known to increase endogenous kinins, mimicked BT venom action (2,6). The cardiopulmonary reflexes are evoked by 5-HT agonists, stimulating the vagal afferents involving 5-HT$_3$ type of receptors (9,19). Kinins and other agents (prostaglandins, histamine, etc) are reported to sensitize the regenerative region of vagal C fibers so as to increase the reflex activity (16). But sensitization of these 5-HT$_3$ receptors by kinin is not known. This study was performed to determine the sensitization of 5-HT$_3$ receptors by venom and compared with that produced by exogenously administered bradykinin.

MATERIALS AND METHODS

Animals, anesthesia, and recording procedure

Adult rats (150-250 g) of either sex of the Charles Foster strain were anaesthetized with urethane (1.5 g/kg, IP). The trachea, right jugular vein, and femoral artery were cannulated. Recordings of arterial blood pressure (femoral artery cannula connected to Stathum transducer), respiratory movements (by force displacement transducer), and electrocardiographic potentials (needle electrode in standard limb lead II configuration) were recorded. The

animals were allowed to stabilize for 30 min after surgical procedures.

Experimental protocol

The bradycardiac response was obtained for > 60 s after the injection of 5-HT. The time-response area of 5-HT-induced bradycardia was computed as mentioned earlier (2,22) to obtain the concentration-response curve of 5-HT (5-100 µg/kg). The same 5-HT concentrations were repeated 30 min after intravenous injection of BT venom (20 µg/kg). Not more than three concentrations of 5-HT were tested in a given experiment. A minimum interval of 10 min was allowed between each 5-HT concentration. Submaximal concentration of 5-HT (25 µg/kg) was chosen for subsequent experiments where all the three parameters were studied.

In the venom only group, 5-HT (25 µg/kg, IV) response was obtained before and 30 min after the injection of BT venom (20 µg/kg, IV). BT venom concentration used in this study was sub-lethal (21). In the aprotinin-treated group, 5-HT response was obtained before (initial response), 10 min after aprotinin (6000 kallikrein inactivating unit, KIU bolus, IV), and 30 min after venom (20 µg/kg). In the ondansetron-treated group, 5-HT response was obtained before (initial response), 15 min after ondansetron (10 µg/kg, IV), and 30 min after venom. In the bradykinin-treated group, 5-HT response was obtained before (initial response), 30 min after bradykinin (50 µg/kg, IV), and 15 min after ondansetron.

Drugs and solutions

Crude BT venom was obtained from the Haffkine Institute, Bombay, India. Bradykinin was obtained from Bachem Feinchemikalien AG, Budendrof, Switzerland, and 5-HT creatinine sulfate from Sigma Chemical Co, St. Louis, MO, USA. Aprotinin was obtained from Wako Pure Chemical Industries Ltd., Japan, and Ondansetron from Natik pharmaceuticals, Bombay, India. A stock solution of all the drugs was prepared in distilled water, and subsequent dilutions were made in normal saline at the time of administration.

Statistical analysis

Data were presented as mean ± SE values. The values of mean arterial pressure (MAP), heart rate (HR), and respiratory rate (RR) before the injection of 5-HT were taken as control response, and the values after the injection of 5-HT at every 5 s, were normalized to the control response. The values at corresponding time interval were pooled to obtain mean ± SE. The time-response area elicited by 5-HT after venom or bradykinin were normalized to the before injection values. The differences between the groups were compared by ANOVA or Student's *t*-test as required. A p value < 0.05 was considered significant.

RESULTS

BT venom shifted the concentration-response curve of 5-HT to the left

5-HT produced a concentration-dependent increase in bradycardiac response area. The maximum response occurred at 50 µg/kg of 5-HT. After exposure to BT venom (20 µg/kg), the 5-HT concentration-response curve was shifted to the left between 5-25 µg/kg (Figure 1; $p < 0.05$; two way ANOVA). No response was observed at 5 µg/kg of 5-HT before venom exposure, but after venom, a significantly greater response was observed at the same concentration (Figure 1). The concentration producing maximal response remained unaltered after venom exposure. Hence, a submaximal concentration of 5-HT (25 µg/kg) was used for subsequent experiments.

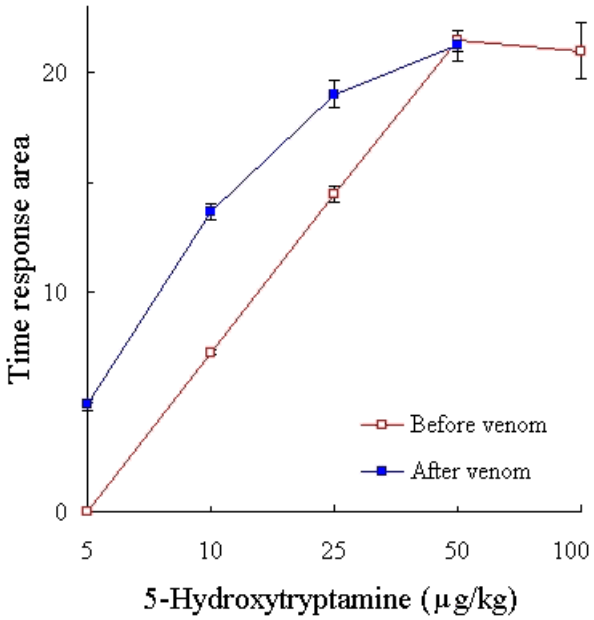

Figure 1. The concentration-response curve of 5-HT reflex was shifted to the left after 30 min exposure to BT venom (20 µg/kg, IV). Mean ± SE values (n = 5-7) between 5-25 µg/kg were significantly different from before

venom injection ($p < 0.05$, two way ANOVA).

5-HT-induced reflex responses were augmented by BT venom

5-HT (25 µg/kg) evoked hypotension, bradycardia, and apnea, extending over a period of time. After venom administration (20 µg/kg, IV, 30 min), all these responses (magnitude as well as time period) were greatly augmented (Figure 2, Figure 3, Figure 4 and Figure 5; $p < 0.05$, ANOVA). The resting values of MAP, HR, and RR before venom injection were 95 ± 5.7 mm Hg, 286 ± 18 per min, and 84 ± 15.6 per min, respectively. After venom, no significant changes in MAP, HR, and RR were observed from before injection values.

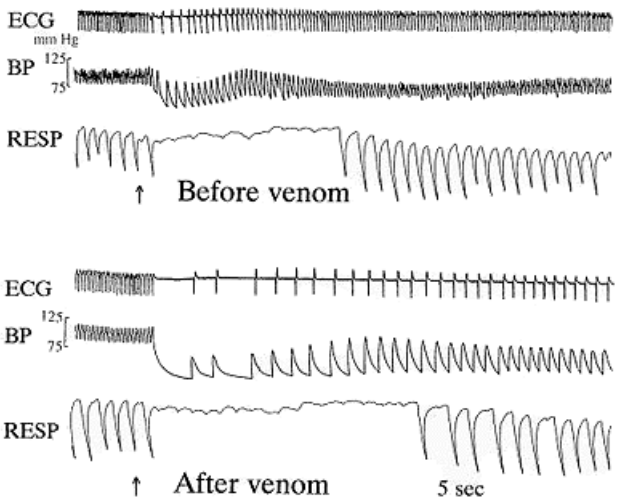

Figure 2. The original tracings of 5-HT-induced changes in heart rate (from ECG), blood pressure (BP), and respiratory movements (RESP) before and 30 min after BT venom (20 µg/kg, IV) from a single experiment are shown. The arrows indicate the point of injection of 5-HT (25 µg/kg, IV) in each panel.

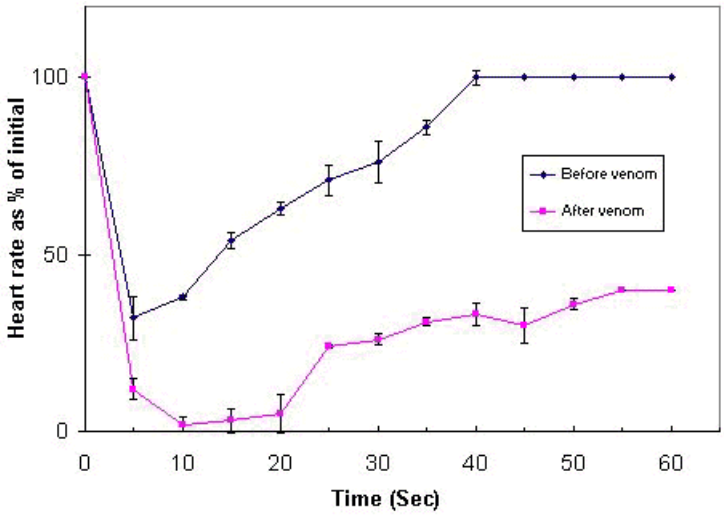

Figure 3. 5-HT-induced reflex changes in heart rate before and 30 min after venom (20 µg/kg, IV). The responses at every 5 s were normalized to the initial values before injection of 5-HT (25 µg/kg). After venom, the responses are significantly different from the before injection values ($p < 0.05$, two way ANOVA). Each point indicates the mean ± SE values from 6 different experiments and error bars at many places are within the symbols.

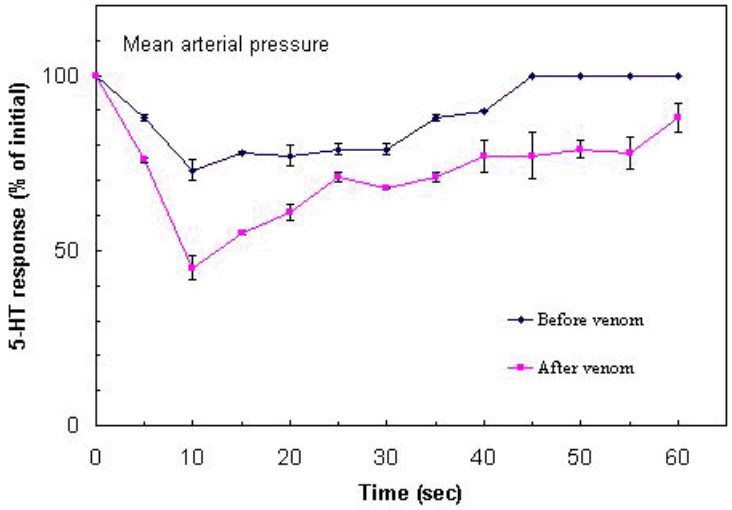

Figure 4. 5-HT-induced reflex changes in mean arterial pressure before and 30 min after venom (20 µg/kg, IV).

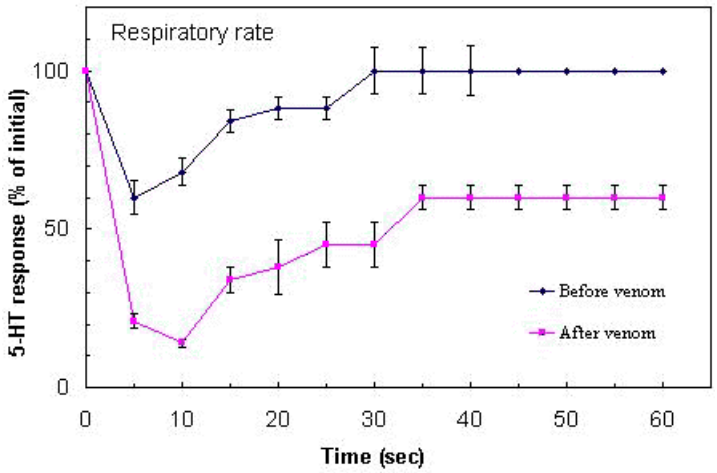

Figure 5. 5-HT-induced reflex changes in respiratory rate before and 30 min after venom (20 µg/kg, IV).

Aprotinin blocked reflex augmentation

Pretreatment with aprotinin (6000 KIU) did not alter the magnitude of the cardiopulmonary reflexes elicited by 5-HT (Figure 6). In aprotinin-pretreated animals, the parameters remained unaltered even after venom exposure. The resting values of MAP, HR, and RR remained unaltered after aprotinin, and after aprotinin and venom.

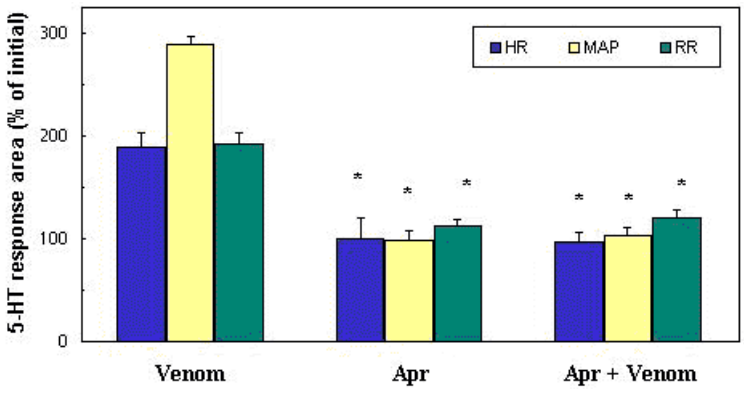

Figure 6. Histograms depict the mean ± SE values (n =6) of the time-response area of 5-HT responses (heart rate - HR, mean arterial pressure - MAP, and respiratory

rate - RR) in venom only group (20 µg/kg, IV), 10 min after aprotinin (Apr; 6000 KIU), and 30 min after venom in Apr-treated animals. * = $p < 0.05$ as compared to venom (unpaired *t* test).

Ondansetron completely blocked the reflex and venom reversed it partially

Pretreatment with ondansetron (10 µg/kg) completely blocked the 5-HT reflex response (Figure 7). After 30 min of venom exposure, around 80% of the initial reflex response re-appeared (Figure 7). The resting values of MAP, HR, and RR were not altered after ondansetron, and after ondansetron and venom.

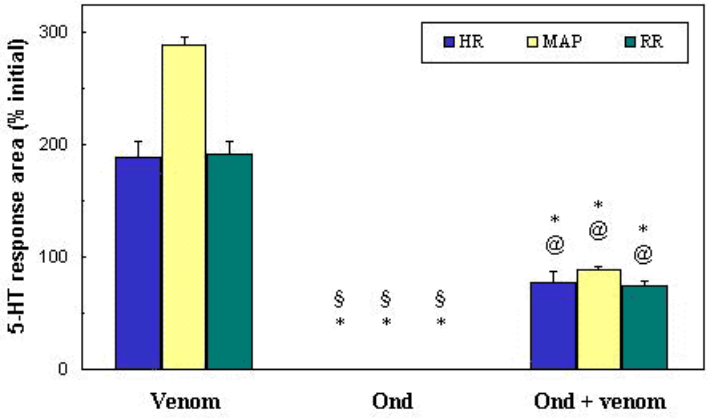

Figure 7. Histograms depict the mean ± SE values (n =6) of the time-response area of 5-HT responses in the venom only group (20 µg/kg, IV), 15 min after ondansetron (Ond, 10 µg/kg), and 30 min after venom in Ond-treated animals. * = $p < 0.05$ as compared to venom (unpaired *t* test); § = $p < 0.05$ as compared to initial response (paired *t* test); @ = $p < 0.05$ as compared to ondansetron (paired *t* test). Note the reappearance of reflex after venom exposure.

Bradykinin mimicked venom action in augmenting the reflex response

Bradykinin (50 µg/kg) augmented the 5-HT-induced reflex response similar to that seen with BT venom (Figure 8). The augmentation of the 5-HT-induced reflex observed after bradykinin was blocked by ondansetron (10 µg/kg, IV, Figure 8). The values after ondansetron were significantly different from those before ondansetron (Figure 8; $p < 0.05$; Students *t* test for paired observations). After bradykinin, resting MAP and RR were not altered, but HR decreased by 32% of before injection values ($p < 0.05$, paired *t* test).

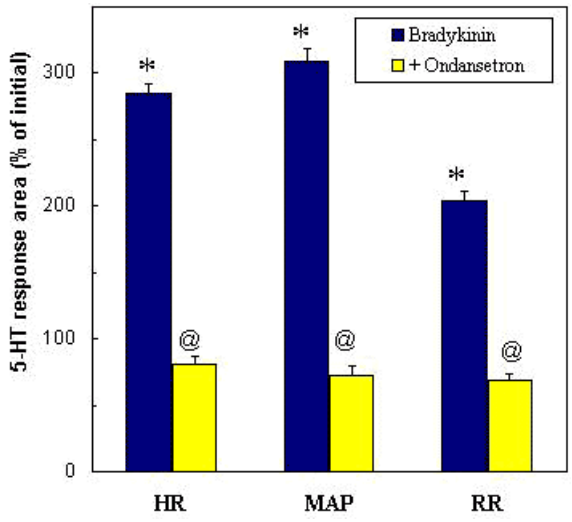

Figure 8. The 5-HT-induced reflex responses (mean ± SE; n = 5) are presented after 30 min exposure to

bradykinin (50 µg/kg) and 15 min after ondansetron (10 µg/kg) in the same animal. * = $p < 0.05$ as compared to initial response (paired *t* test); @ = $p < 0.05$ as compared to the response after bradykinin.

DISCUSSION

The results of this study indicate that BT venom augmented the 5-HT reflexes similar to phenyldiguanide (2,22). The blockade of venom-induced augmentation by kinin synthesis inhibitor, aprotinin, as well as the augmentation of the reflex by exogenously administered bradykinin both reveal the role of kinins in venom action. Further, in the previous study, captopril, an agent known to increase the endogenous kinins, augmented the cardiopulmonary reflexes to the same magnitude (2,6), supporting the above point. Thus, exogenous or endogenous kinins augment the reflex parameters.

5-HT_3 receptor antagonist blocked the 5-HT agonist-induced cardiopulmonary reflexes both in this study and elsewhere (9,19). In this study, however, after venom exposure, the reflex re-appeared. Re-appearance of the reflex after venom or failure to completely block the reflex by ondansetron (Figure 6 and Figure 8) indicate sensitization of 5-HT_3 receptors. Sensitization of the regenerative regions of the sensory receptors by inflammatory mediators, such as prostaglandins, kinins, histamine, etc have been indicated (16). This sensitization was demonstrated experimentally with histamine (1). The present results suggest that BT venom augments the cardiac reflexes by sensitizing the 5-HT_3 receptors involving kinin mechanisms.

Kinins are peptides in nature and act on the cell surface receptors (B_1 and B_2) to evoke biological responses (18). The B_2 receptors are stable cell membrane receptors, while B_1 receptors are silent in normal conditions, but can be activated by noxious chemical stimuli (11,18). The *de novo* formation of B_1 receptors has also been reported after tissue injury/inflammation/after lipopolysaccharide injection (4,5,7,10). Similarly, the activation of B_1 receptors can be proposed for producing the augmentation of cardiopulmonary reflexes seen with venom or bradykinin in this study. In addition, bradykinin also increases the capillary permeability, which in turn produces edema. The natural stimulant proposed for the activation of cardiopulmonary reflexes is pulmonary edema (1,17). Pulmonary edema is one of the common manifestations of scorpion stings (3,14). Thus, this supports the pulmonary edema as the cause of the reflex augmentation after scorpion toxicity.

In conclusion, the augmentation of cardiopulmonary reflexes either by bradykinin or venom involves 5-HT_3 receptor subtypes for their action. The results further indicate that venom action is mediated through kinins via sensitization of 5-HT_3 receptors to increase the reflex response.

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