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Oleic Acid-Induced Pulmonary Edema Did Not Augment the Vagal C-Fiber Reflexes Elicited by Phenylbiguanide and Capsaicin as Seen with *Mesobuthus tamulus* Venom.

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ABSTRACT

Vagal C-fiber reflexes elicited by phenylbiguanide (PBG) and capsaicin are sensitized by *Mesobuthus tamulus* (MBT) venom-induced pulmonary edema or inflammation. Interestingly, pulmonary edema and inflammation are also induced by oleic acid (OA). The present study was undertaken to ascertain whether OA augments the PBG or capsaicin-induced reflexes as seen with MBT venom. Experiments were performed on anesthetized adult rats. Respiratory excursions, blood pressure and ECG were recorded. At the end of each experiment pulmonary water content was determined. PBG (10 µg/kg) or capsaicin (10 µg/kg) produced hypotension, bradycardia and apnea-bradypnea. MBT venom (100µg/kg) augmented PBG as well as capsaicin-induced responses and produced pulmonary edema. OA (30 µl) did not augment PBG or capsaicin-induced responses but produced pulmonary edema. The present results indicate that PBG or capsaicin responses are not augmented in response to OA unlike MBT venom. It is possible that the discrete mechanisms are involved in OA and MBT venom- induced pulmonary pathology.

Keywords: Acute lung injury; Indian red scorpion venom; Phenylbiguanide; Pulmonary C reflexes

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INTRODUCTION

The respiratory alterations produced by *Mesobuthus tamulus* (MBT) venom are comparable to oleic acid (OA) model of ARDS [1]. The respiratory alterations and pulmonary pathology seen after MBT venom or OA included hypoxemia, ventilatory changes, pulmonary inflammation and pulmonary edema [1]. Pulmonary congestion and pulmonary edema are natural stimuli for J-receptors (juxta-pulmonary capillary receptors) located in the pulmonary interstitium [2, 3]. Impulses from J-receptors, also known as vagal C-fiber receptors are carried by the vagal C (unmyelinated) afferents. Agents like phosgene, alloxan or MBT venom have been shown to increase the pulmonary permeability producing pulmonary edema which sensitizes the vagal C-fibers [2-5]. The pulmonary edema induced by MBT venom has been shown to augment the vagal C-fiber reflexes elicited by 5-HT agonists like phenyldiguanide (PDG) or phenylbiguanide [PBG; 6-9]. The augmentation of these reflexes was shown to be mediated through pulmonary edema-dependent mechanisms involving inflammatory mediators like histamine, kinins, nitric oxide (NO), prostaglandins etc. and intracellular signaling molecules like guanylate cyclase, cyclic guanosine monophosphate (cGMP), phospholipase A2 etc. [8-12].

In addition to pulmonary edema, the vagal afferents can be directly sensitized by inflammatory mediators. Recently, it has been shown that MBT venom-induced augmentation of capsaicin reflexes is independent of pulmonary edema [10]. It was proposed that direct sensitization of vagal afferents by inflammatory mediators like prostaglandins released after MBT envenomation produced the augmentation of capsaicin reflexes [10]. Thus, MBT venom can augment the vagal C-fiber reflexes in response to pulmonary edema or inflammatory mediators.

Pulmonary edema and inflammation are also induced by OA [13]. However, the effect of OA on vagal C-fiber reflexes is not known. Considering the similarities in MBT venom and OA-induced pulmonary pathology, we hypothesized that OA may also produce reflex augmentation as reported with MBT venom [6-12]. Therefore, in the present study, effect of OA on vagal C-fiber reflexes elicited by PBG or capsaicin was delineated. Further, they were compared with MBT venom-induced augmentation of PBG and capsaicin reflexes.

MATERIALS AND METHODS

Animals, anesthesia and recording procedure

Experiments were performed according to the guidelines of the ethical committee of Institute of Medical Sciences, Banaras Hindu University, Varanasi, India for conducting animal experiments. Adult female rats (186 ± 21 g) of Charles Foster strain were used. The animals were anaesthetized with urethane (1.5 g/kg i.p). An additional dose of urethane (0.1-0.15 g/kg i.p) was injected if required. Trachea, jugular vein and femoral artery were cannulated. Tracheal cannulation was used to keep the respiratory tract patent; jugular venous cannulation for drug administration; and femoral artery cannulation for recording blood pressure via pressure transducer. Electrocardiographic potentials were recorded by connecting the needle electrodes in standard limb lead-II configuration. Respiratory movements were recorded by securing a thread to the skin over xiphisternum and connecting it to a force-displacement transducer. All the recordings were made on a chart recorder.

Experimental protocol

Dose of PBG or capsaicin (10 µg/kg) was used as reported earlier [8-10]. The animals were divided into 2 groups. Each group was sub-divided into PBG and capsaicin groups.

Group I- In this (MBT venom) group, the effect of MBT venom on PBG and capsaicin response was ascertained. In one subgroup (n = 6), after obtaining the initial PBG (10 µg/kg) reflex response, MBT venom (10 mg/kg) was injected and 30 min later, PBG responses were recorded. In another subgroup (n = 5), same protocol was followed replacing PBG with capsaicin (10 µg/kg).

Group II- In this (OA) group, the effect of OA on PBG and capsaicin response was ascertained. In one subgroup (n = 4), after obtaining the initial PBG (10 µg/kg) reflex response, OA (30 µl) was injected and 30 min

later, PBG reflex response was obtained again. In another subgroup (n = 3), same protocol was followed replacing PBG with capsaicin (10 µg/kg).

Determination of pulmonary water content

The pulmonary water content was determined by the method described earlier [7]. Briefly, at the end of each experiment the lungs were excised, weighed and dried to a constant weight in an electric oven (at 90º C for 48 h). The difference between wet weight and dry weight was calculated to determine the water content.

Drugs and Solutions

Phenylbiguanide (PBG) and capsaicin were obtained from Sigma Chemical Company St. Louis, MO, USA. MBT venom was procured from Irula Co-operative society, Chennai, India and OA was obtained from Hi-media Laboratories Pvt. Ltd, Goa, India. Stock solutions of PBG and MBT venom (1 mg/ml) were prepared in distilled water. Stock solution of capsaicin (1 mg/ml) was prepared in ethanol. Subsequent dilutions of these drugs were made with normal saline at the time of administration. The volume of injections was kept at 0.1 ml.

Analysis of data

The changes in mean arterial pressure (MAP), heart rate (HR), respiratory frequency (RF), in the first 15 s after PBG/capsaicin were calculated and these values were normalized to the initial (before PBG/capsaicin) as 100%. The responses after MBT venom/OA were expressed as % decrease as compared to the initial values (before PBG/capsaicin; 9). The data from various experiments were pooled to obtain mean ± SEM. The effect of MBT venom or OA was compared by using one-way ANOVA. Student's t test for unpaired observations was performed for comparing pulmonary water content. A $P < 0.05$ was considered significant.

RESULTS

MBT venom augmented both PBG and capsaicin-induced reflex response

PBG (10 µg/kg; n = 6) produced apnea-bradypnea, bradycardia and hypotension (Fig. 1A and 1C). MBT venom (100 µg/kg) *per se* did not alter the basal HR, RF and MAP values (Table 1). However, the PBG responses after MBT venom, were significantly augmented (Fig 1A, 1B and 1C; $P < 0.05$, two way ANOVA).

Capsaicin (10 µg/kg; n = 5) produced apnea-bradypnea, bradycardia and hypotension (Fig 1D and 1F). In this group also MBT venom (100 µg/kg) *per se* did not alter the basal HR, RF and MAP values (Table 1). However, capsaicin responses after MBT venom were also significantly augmented (Fig 1D, 1E and 1F; $P < 0.05$, two way ANOVA).

OA did not augment PBG or capsaicin-induced reflex response

In this group also, PBG (10 µg/kg; n = 4) produced apnea-bradypnea, bradycardia and hypotension (Fig. 2A and 2C). OA (30µl) produced tachypnea (Table 1). The PBG responses after OA were similar to the initial PBG responses (Fig. 2A, 2B and 2C).

Capsaicin produced apnea-bradypnea, bradycardia and hypotension (Fig. 2D and 2F). OA (30µl) produced tachypnea (Table 1). The capsaicin responses after OA also remained similar to the initial capsaicin responses (Fig 2D, 2E and 2F).

Pulmonary edema was seen after MBT venom or OA administration

The pulmonary water content in PBG and capsaicin groups receiving MBT (100µg/kg) venom was 79.5 ± 0.18% and 79.6 ± 0.2%, respectively. The pulmonary water content in PBG and capsaicin groups after OA (30µl) was also similar to MBT venom group (Fig. 3A and 3B). These values are significantly greater than basal values of pulmonary water content as reported elsewhere (76.3 ± 0.2 %; $P < 0.05$ Student's t-test for unpaired observations; 10).

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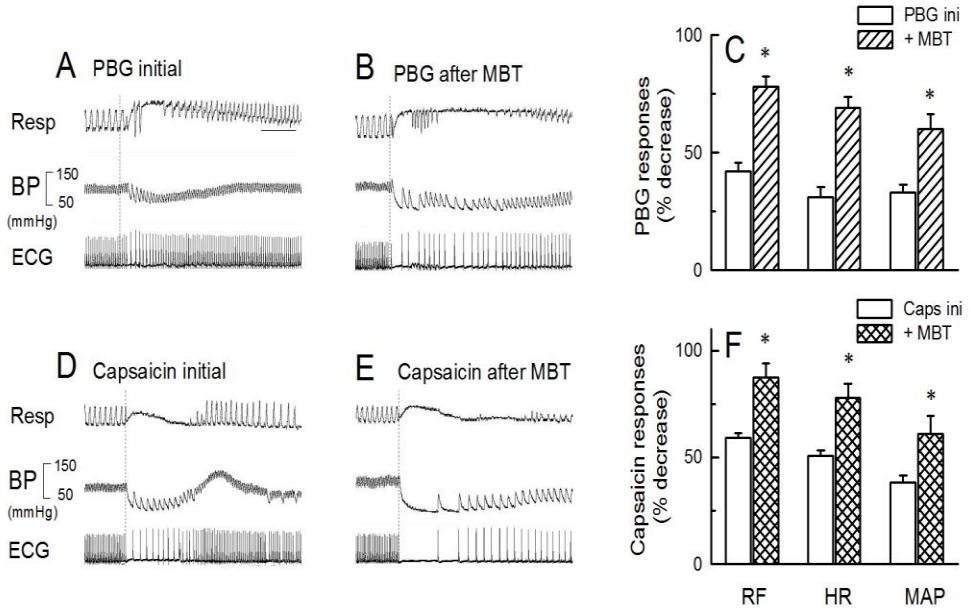

Fig 1: MBT venom augmented the apneic-bradypneic, bradycadiac and hypotensive responses elicited by PBG or capsaicin. In A and B, the original tracings of an experiment showing the PBG (10 µg/kg)-induced responses before and 30 min after MBT venom (100 µg/kg) are presented, respectively. In D and E, the capsaicin (10 µg/kg)-induced responses before and 30 min after MBT venom (100 µg/kg) are presented, respectively. Electrocardiogram, ECG; respiration, Resp and blood pressure, BP in mm Hg. Vertical dashed line indicates the point of injection of PBG/capsaicin and horizontal line indicates time scale = 10 s for all. Histograms in C and F show PBG and capsaicin responses before and 30 min after MBT venom, respectively. Each bar represents the mean ± SEM values from 5-6 experiments in each group. An asterisk (*) in C and F indicates P < 0.05 as compared to the initial responses (two-way ANOVA).

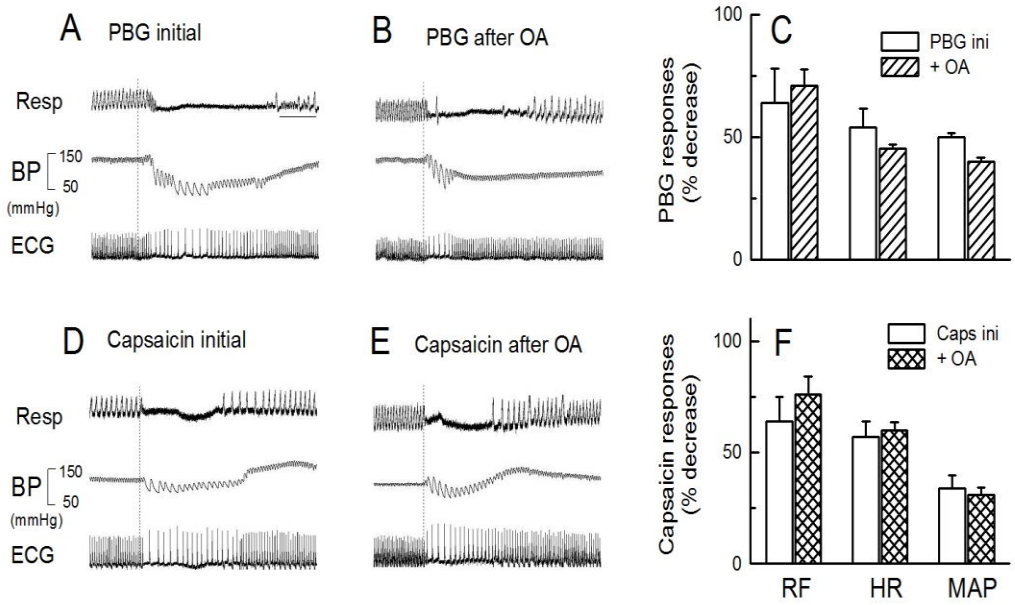

Fig 2: OA did not augment the apneic-bradypneic, bradycadiac and hypotensive responses elicited by PBG or capsaicin. In A and B, the original tracings of an experiment showing the PBG (10 µg/kg)-induced responses before and 30 min after OA (30 µl) are presented, respectively. In D and E, the capsaicin (10 µg/kg)-induced responses before and 30 min after OA (30 µl) are presented, respectively. Electrocardiogram, ECG; respiration, Resp and blood pressure, BP in mmHg. Vertical dashed line indicates the point of injection of PBG/capsaicin and horizontal line indicates time scale = 10 s for all. Histograms in C and F show PBG and capsaicin responses before and 30 min after OA (30 µl), respectively. Each bar represents the mean ± SEM values from 3-4 experiments in each group.

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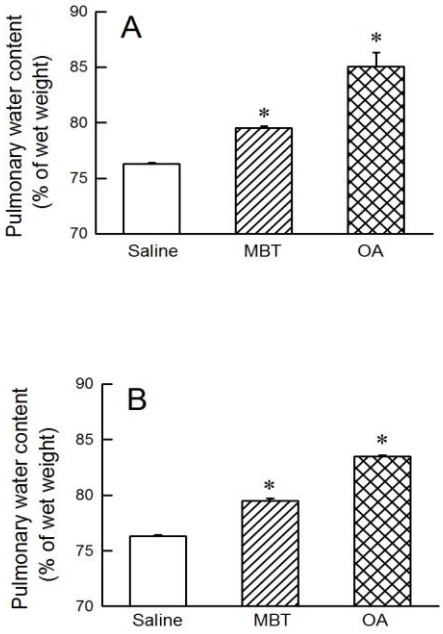

Fig 3: The pulmonary water content was increased after MBT or OA administration. Histograms in A and B show the values of pulmonary water content in PBG and capsaicin groups respectively after MBT venom (MBT)/oleic acid (OA) administration. The values for pulmonary water content in saline group are taken from earlier report [10]. Each bar represents the mean ± SEM values from 3 to 6 different experiments. An asterisk (*) indicates P < 0.05 as compared to the saline groups (Student's t test for unpaired observations).

Table 1: Baseline values of MAP, RF and HR in phenylbiguanide (PBG) and capsaicin groups before and after saline/MBT/OA administration.

Group	PBG			Capsaicin		
	HR	RF	MAP	HR	RF	MAP
MBT venom group (n = 6)				***MBT venom group (n = 5)***		
Initial	249 ± 16.0	84.0 ± 6.0	82.3 ± 11.0	233 ± 27	81.6 ± 3.8	69.5 ± 4.6
+MBT	205 ± 15.9	80.0 ± 2.0	76.3 ± 10.0	204 ± 34	84.0 ± 2.4	74 ± 7.3
OA group (n = 4)				***OA group (n = 3)***		
Initial	276 ± 32.1	92 ± 10.5	72.2 ± 6.9	276 ± 48.0	94 ± 5.3	80.3 ± 10.9
+OA	207 ± 18.5	180 ± 12.9	69.0 ± 14.0	240 ± 36.0	150 ± 21.0	79.0 ± 10.0

The values are mean ± standard errors from 'n' number of experiments in each group. MAP, mean arterial pressure; RF, respiratory frequency (per minute) and HR, heart rate (beats per minute; bpm).

DISCUSSION

The present work was aimed to determine whether OA also augments the PBG or capsaicin-induced reflex responses similar to MBT venom. Our results indicate that OA produced pulmonary edema but failed to augment the vagal C-fiber reflexes elicited by PBG or capsaicin as seen with MBT venom.

Vagal C-fiber reflexes can be elicited by agents like PBG, PDG, capsaicin etc. [4, 6-12]. These reflexes are augmented in response to pulmonary congestion or pulmonary edema [2, 3]. MBT venom-induced pulmonary edema has been shown to augment the vagal C-fiber reflexes elicited by 5HT agonists like PBG/PDG involving intracellular signaling mechanisms like kinin mediated NO-GC-cGMP pathway or PLA2 pathway [6, 11,12]. Corresponding to the earlier reports, MBT venom produced pulmonary edema and augmented the PBG-induced reflex responses in this study also (Fig 1A, 1B, 1C and 3A). On the other hand, OA produced pulmonary edema that was comparable to MBT venom but did not augment the PBG-induced reflex response (Fig 2A, 2B, 2C and Fig 3A).

Vagal C-fiber reflexes can also be augmented by pulmonary edema-independent mechanisms. Recently, pulmonary edema-independent mechanisms have been shown to augment the capsaicin-induced reflexes by MBT venom (10). The capsaicin-induced reflexes are mediated through the TRPV1 receptors. TRPV1 receptors are ligand gated non-selective cation channels that are sensitive to large number of regulatory factors including heat, acid, inflammatory mediators, arachidonic acid derivatives etc (10,14). Involvement of prostaglandins for the augmentation of capsaicin-induced reflex responses by MBT venom has been reported (10). Similar to MBT venom, severe inflammation is also produced by OA (1). OA is known to damage the pulmonary endothelium producing inflammation (1,15). Thus, it is expected that OA would augment the capsaicin-induced vagal C-fiber reflexes involving pulmonary-independent mechanisms. However, OA did not augment the capsaicin-induced reflexes (Fig 2D, 2E and 2F). Thus, OA did not augment the vagal C-fiber reflexes elicited either by PBG (pulmonary edema-dependent) or capsaicin (pulmonary edema-independent), as reported for MBT venom.

The inability of OA to augment the PBG or capsaicin-induced responses despite producing pulmonary edema and inflammation may be due to damage of the vagal C-fibers or its receptors by OA. However, the PBG/capsaicin responses seen after OA were similar to the initial responses (before OA; Fig. 2D, 2E and 2F) which rules out the possibility of damage to the reflex pathway (vagal C-fibers/receptors). It is to note that vagal C-fiber reflexes in response to pulmonary congestion, pulmonary edema and pulmonary hypertension manifest to decreased ventilation and perfusion. On the other hand, OA-induces hypoxemic drive which manifests as increased ventilation and perfusion [1, 13]. Thus, in OA challenged animals there is conflict between two opposing physiological responses i.e the hypoxemic drive and pulmonary edema/congestion drive. Since it is very difficult to isolate these mechanisms, it is anticipated that hypoxemic drive might have masked the reflex effects of OA-induced pulmonary edema or inflammation. Hence, OA-induced augmentation of reflexes was not apparent in the present study. Supporting this proposition, the augmentation of pulmonary nociceptor activity has been shown after OA (16). Since MBT venom did not induce a situation like hypoxemic drive, the augmentation of PBG/capsaicin-induced reflexes was evident in this group (1). These findings support the proposition of differences in pathophysiological mechanisms underlying OA-induced or MBT venom-induced respiratory changes (1).

CONCLUSION

The vagal C-fiber reflexes elicited by PBG or capsaicin are augmented in response to MBT venom but not by OA. The hypoxemic drive induced by OA (but not by MBT venom) appears to mask the effect of OA on reflexes produced by PBG or capsaicin. It is also proposed that the hypoxemic drive plays a dominant role over vagal C-fiber reflexes (induced by pulmonary edema/congestion) in mediating OA-induced respiratory alterations.

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