Indian J Physiol Pharmacol 2014; 58(4) : 312–318

Original Article

Pulmonary oedema producing toxin from *Mesobuthus tamulus* venom augments cardio-respiratory reflexes through B_2 kinin receptors

Anitha B. Alex[1]*, Aparna Akella, Anil K. Tiwari and Shripad B. Deshpande*

Department of Physiology,
Institute of Medical Sciences,
Banaras Hindu University,
Varanasi – 221 005, India

Abstract

The current study was undertaken to compare the effects of pulmonary oedema producing toxin (PO-Tx) isolated from *Mesobuthus tamulus* venom on cardio-respiratory reflexes with exogenously administered bradykinin (BK) and to delineate the type of BK receptors mediating these responses. Jugular venous injection of phenyldiguanide (PDG) in anaesthetized rats produced reflex bradycardia, hypotension and apnoea. The PDG-induced reflex was augmented (two folds) by PO-Tx. The pulmonary water content in PO-Tx treated group was also increased. The PO-Tx-induced reflex changes as well as pulmonary oedema were blocked by Hoe-140 implicating the involvement of B_2 kinin receptors. Exogenous BK also produced augmentation (two folds) of the PDG-induced reflexes and increased the pulmonary water content. The BK-induced augmentation was blocked by pre-treatment with des-Arg^{10} Hoe 140 (a B_1 receptor antagonist) and Hoe 140 (B_2 receptor antagonist). However, these antagonists did not prevent the development of BK-induced pulmonary oedema. Present results indicate that PO-Tx augmented the PDG-induced reflex responses similar to BK and the PO-Tx induced augmentation of reflexes is mediated through B_2 receptors.

Introduction

Pulmonary oedema, a fatal manifestation of scorpion envenomation was given relatively less attention; often priority was given to the cardiovascular abnormalities (1-3). In recent years, a number of non-cardiogenic factors have been implicated for pulmonary oedema formation after scorpion toxicity. They include: direct toxicity of venom on pulmonary tissues; venom induced release of inflammatory mediators that affect capillary permeability; metabolic alterations and ischemia/hypoxemia that can lead to surfactant insufficiency (4-9). Inflammatory mediators such as kinins have been reported to be crucial for regulating capillary permeability and pulmonary oedema after scorpion envenomation (10-16). Increased bradykinin levels in animals injected with *Leiurus quinquestriatus quinquestriatus* and *Tityus serrulatus* venom has been reported (12,17). In addition, bradykinin potentiating peptides were isolated from the venom of different species of scorpions (18-20). Further, the role of

***Corresponding author :**
Prof. S. B. Deshpande, Department of Physiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221005, India, Tel.: 91-542-2369069; Fax:91-542-2367568; E-mail:desh48@yahoo.com

[1]Present address :
Anticonvulsant Drug Development Program, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City UT, USA.

(Received on June 29, 2013)

kinins and kinin receptors in *Mesobuthus tamulus* (MBT) venom-induced pulmonary oedema formation has been demonstrated (5, 10, 11, 21). A newly isolated toxic fraction (Pulmonary Oedema producing toxin or PO-Tx) from MBT venom has been shown to be responsible for the production of pulmonary oedema after scorpion envenomation (22).

Pulmonary oedema is the natural stimulus for the activation of vagal C fibers (23, 24). These fibers can also be stimulated by kinins, prostaglandins and other endogenous substances (23, 24). Intravenous injection of phenyldiguanide (PDG) stimulates the vagal C fibers located in heart and lungs and produces a reflex bradycardia, hypotension and apnoea-tachypnoea (23, 24). The PDG-induced reflex response has been shown to be augmented by MBT venom/PO-Tx involving kinins (10, 11, 22). Since, kinin synthase inhibitor blocked the augmentation of PDG-induced responses, it was hypothesized that PO-Tx increases endogenous kinin levels to produce reflex augmentation and pulmonary oedema. The present study was therefore undertaken to compare the actions of PO-Tx with exogenously administered bradykinin. Further, types of BK receptors involved in mediating the action of PO-Tx were also investigated.

Materials and methods

Drugs and solutions

Lyophilized *Mesobuthus tamulus* venom was purchased from Haffkine's Research Institute, Mumbai, India. PO-Tx, a lethal toxic fraction was isolated from crude MBT venom as described elsewhere (22). Briefly, crude MBT venom was subjected to gel filtration chromatography using sephadex G-75 column (diameter 1.5 cm and height 60 cm). The elute fractions between 54-94 ml were again subjected to cation-exchange chromatography on carboxymethyl cellulose column to obtain PO-Tx (22). Hoe 140 and des-Arg^{10}-Hoe 140 (des-Arg) were obtained from Sigma Chemical Co., USA; Bradykinin from Bachem, Switzerland. Phenyldiguanide (PDG) was from Koch Light laboratories, Bucks, U.K. Stock solutions of all the drugs were prepared in distilled water and subsequent dilutions were made in normal saline at the time of administration.

Animals, anaesthesia and recording procedure

The animal experiments were performed after obtaining approval from the Ethical Clearance Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Adult male albino rats (150-250 g) of Charles Foster strain were anaesthetized with urethane (1.5 g/kg; i.p.). Tracheal cannulation was done to keep the respiratory tract patent, followed by jugular venous and femoral arterial cannulations to deliver drugs/antagonists and blood pressure (BP) recordings, respectively. Recordings of ECG, blood pressure and respiration were made on chart recorder. Animals were allowed to stabilize for 30 min after the surgical procedures.

Experimental protocol

Animals were divided into four groups. In all the experimental groups, 10 µg/kg phenyldiguanide (PDG) was injected to obtain the initial PDG response.

In group I (n=6), after the initial PDG response, PO-Tx (50 µg/kg; i.v.) was injected and after 30 min, the PDG response was obtained again. In another set (n=4), time-matched responses after saline were recorded.

In group II (n=5), after taking the initial PDG response, Hoe 140 (B_2 receptor antagonist; 5 nM/kg) was injected and 15 min later, the PDG response was elicited. Subsequently, PO-Tx was injected and after 30 min, the PDG response was obtained again.

In group III (n=4), after obtaining the initial PDG response, bradykinin (50 µg/kg) was injected and after 20 min, the PDG response was elicited again.

In group IV (n=10), after taking the initial PDG response, bradykinin receptor antagonist (des-Arg or Hoe 140; 5 nM/kg) was injected and 15 min later, the PDG response was elicited. Subsequently, bradykinin (50 µg/kg) was injected and after 20 min, the PDG response was obtained again.

Determination of pulmonary water content

Pulmonary water content was detected by the

difference in wet weight and dry weight of the lungs and was expressed as percentage of wet lung tissue, as described previously (5).

Analysis of data and statistics

The time response area of heart rate (HR), respiratory frequency (RF) and mean arterial pressure (MAP) after PDG was calculated, as described earlier (21). The response area after drugs/antagonists was normalized to the initial PDG response area. The pooled data were presented as means±S.E.M. Differences between various groups were compared using Student's *t*-test for paired and unpaired observations as required. A P value < 0.05 was considered significant.

Results

PO-Tx augmented PDG reflex

Injection of PDG produced reflex apnoea, hypotension and bradycardia, as described earlier (Fig. 1A; 10, 11, 22). PDG-induced reflex hypotension, bradycardia and apnoea were potentiated about 2 times after PO-Tx (Fig. 1B and C). There was a significant increase in pulmonary water content in PO-Tx treated group as compared to saline control group (Fig. 2B).

B_2 receptor antagonist blocked the action of PO-Tx

Pre-treatment with Hoe 140, a B_2 receptor antagonist (5 nM/kg) did not alter the basal HR, RF and MAP values. The PDG-induced reflex changes after pre-treatment with Hoe 140 were similar to the initial PDG reflex. However, the augmentation of PDG-induced reflex was not seen after PO-Tx in Hoe 140 pre-treated animals (Fig. 2A). PO-Tx induced increase in pulmonary water content was also not seen after pre-treatment with Hoe 140 (Fig. 2B).

Exogenous bradykinin (BK) augmented PDG-induced reflex

The resting HR, RF and MAP were increased slightly after BK (50 µg/kg) administration. In BK treated animals, the PDG-induced cardiopulmonary reflex was augmented significantly (Fig. 3A, B and C). There was also increased pulmonary water content in BK

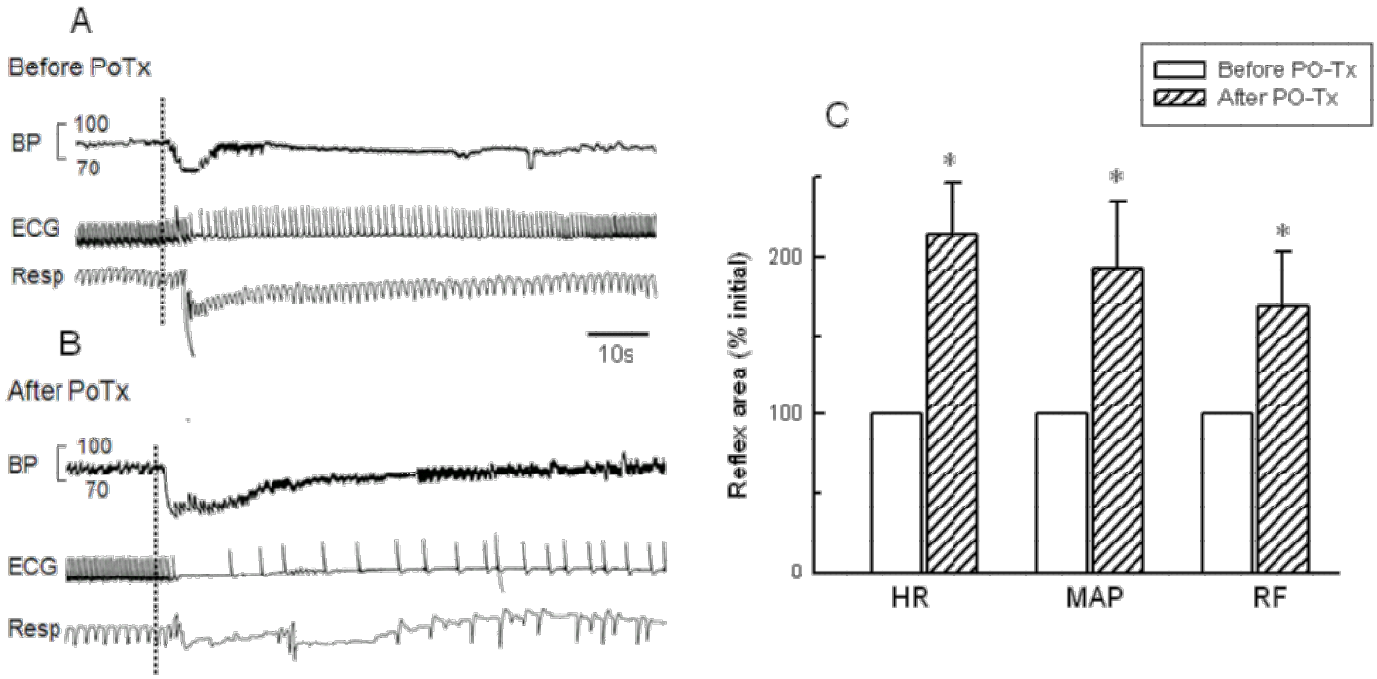

Fig. 1 : Augmentation of PDG (10 µg/kg) induced reflex by PO-Tx. Original tracings of an experiment showing the PDG reflex responses (BP, ECG, and respiration, Resp) before and after administration of PO-Tx (50 µg/kg) are given in A and B, respectively. The dotted line indicates the point of administration of PDG. Time scale = 10s. In C, each bar depicts mean±SEM from 6 experiments showing the reflex changes in HR, MAP and RF obtained initially (Before PO-Tx) and after PO-Tx. An asterisk (*) indicates P < 0.05, as compared to initial PDG response (Before PO-Tx) (Student's t-test for paired observations).

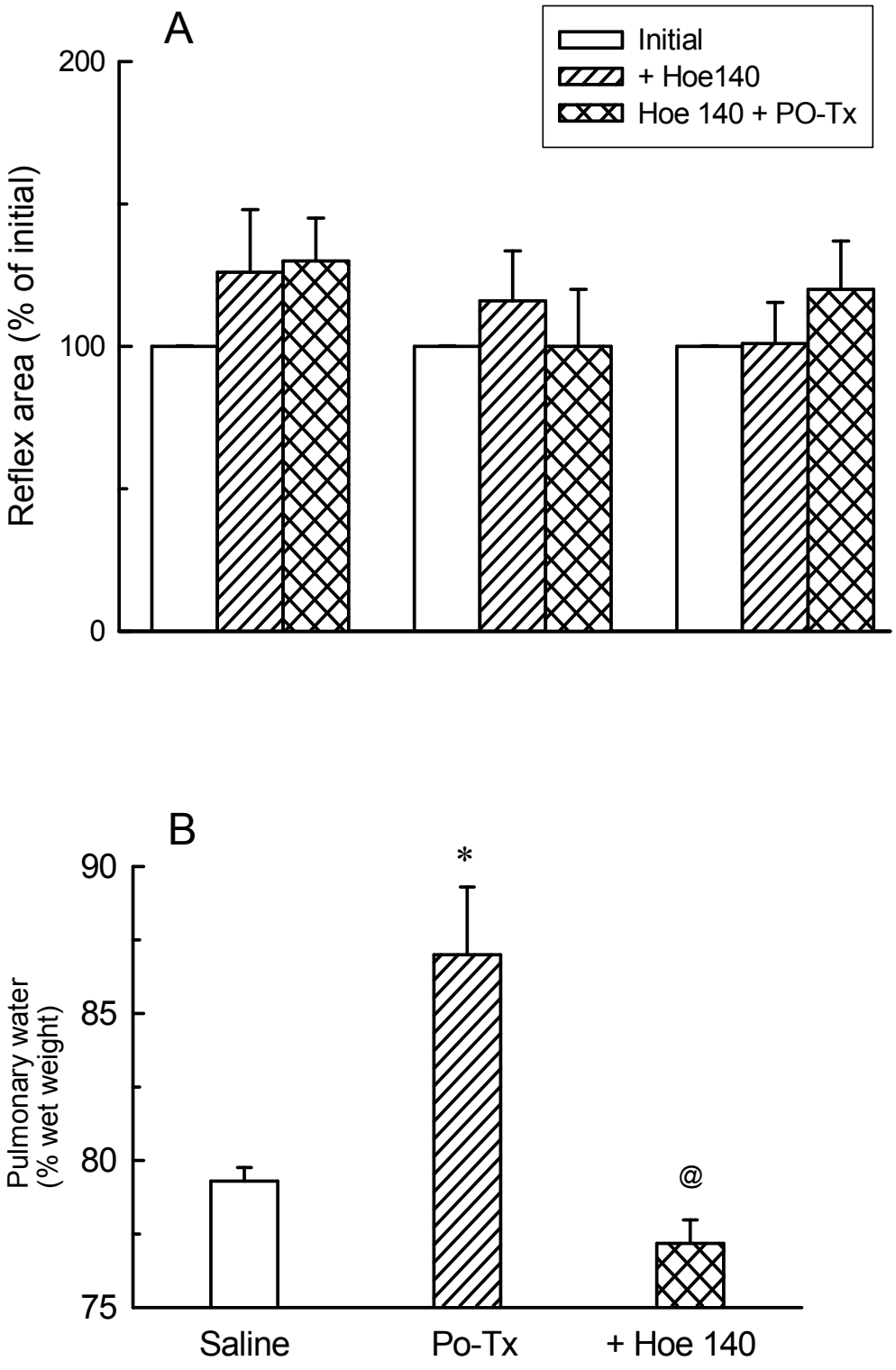

Fig. 2 : Hoe 140 blocked the PO-Tx-induced augmentation of PDG responses. Histogram in A shows the PDG reflex obtained initially (initial), after pretreatment with Hoe 140 (+ Hoe 140) and after PO-Tx (Hoe 140+PO-Tx). In B, the pulmonary water content in saline control, PO-Tx and Hoe 140 pretreated groups are shown. Each bar represents the mean±SEM from 4-6 experiments. An asterisk (*) represents $P<0.05$ as compared to control group and @ represents $P<0.05$, as compared to PO-Tx group (Student's t–test for unpaired observations). Note: PO-Tx-induced augmentation observed in Fig. 1 is not seen after Hoe 140.

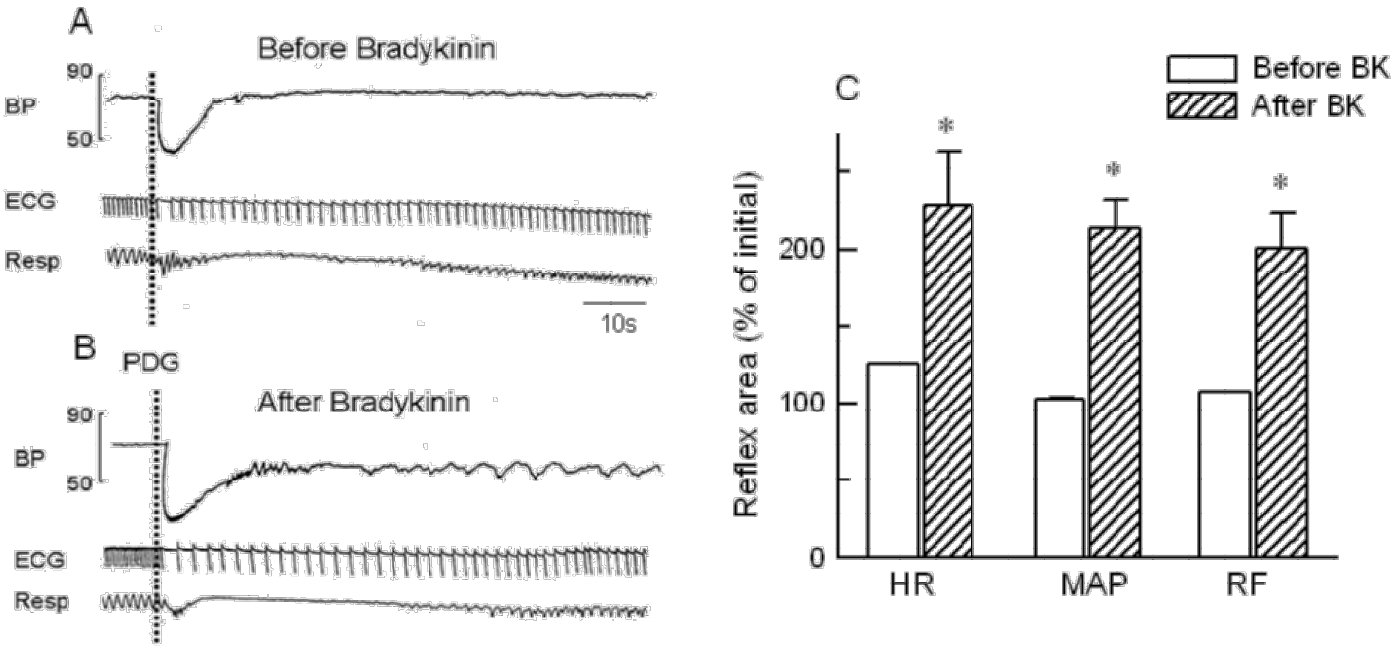

Fig. 3 : Bradykinin (BK) mimics the action of PO-Tx. Figures in A and B show the original tracings of BP, ECG and respiration (Resp) before and after bradykinin (50 μg/kg), respectively. Point of administration of PDG is indicated by the dotted line. Time scale = 10s. In C, the reflex changes in HR, MAP and RF obtained initially (Before BK) and after BK are given. Each bar represents the mean±SEM from 4 experiments. An asterisk (*) indicates, P<0.05, as compared to initial responses (Student's t-test for paired observations).

treated animals as compared to saline control group (Fig. 4C).

B_1 and B_2 kinin receptor antagonists blocked the bradykinin induced augmentation of reflex responses but not the pulmonary oedema

Pre-treatment with B_1 receptor antagonist (des-Arg; 5 nM/kg) or B_2 receptor antagonist (Hoe 140, 5 nM/kg) did not alter the basal HR, RF and MAP values. The magnitude of PDG-induced reflex responses after pre-treatment was similar to the previous responses. The BK-induced reflex augmentation was blocked in both B_1 and B_2 receptor antagonist pre-treated groups (Fig. 4 A and B). On the contrary, BK-induced pulmonary oedema persisted in these groups (Fig. 4 C).

Discussion

Identification of pulmonary oedema producing toxin (PO-Tx) from *Mesobuthus tamulus* venom (22) illustrates the highly specific and selective toxin present in scorpion venom which produces pulmonary oedema and is responsible for the augmentation of PDG-induced reflex response (22). The current results while confirming the previously reported effects of PO-Tx, further reveal that exogenously administered bradykinin also produces similar effect on the cardiorespiratory reflexes. In addition, the action of PO-Tx is mediated through B_2 receptors.

In the earlier studies, kinin synthase inhibitor (aprotinin) blocked the PO-Tx induced responses (22). In the present study, exogenously administered BK augmented the cardio-respiratory reflexes similar to PO-Tx. Further, earlier reports have shown that aprotinin, prolongs the survival time of experimental animals injected with MBT venom (15, 16). Thus, kinins play a vital role in mediating PO-Tx/MBT venom-induced toxicity. Kinins are low-molecular weight inflammatory peptides, originating from kininogens through the action of kallikreins. Kinins mediate their actions through G protein coupled B_1 and B_2 receptors (25). B_1 receptors are expressed in response to inflammation and mediate hyperalgesia (26, 27). B_2 receptors are constitutively expressed and regulate capillary permeability (25). In the present

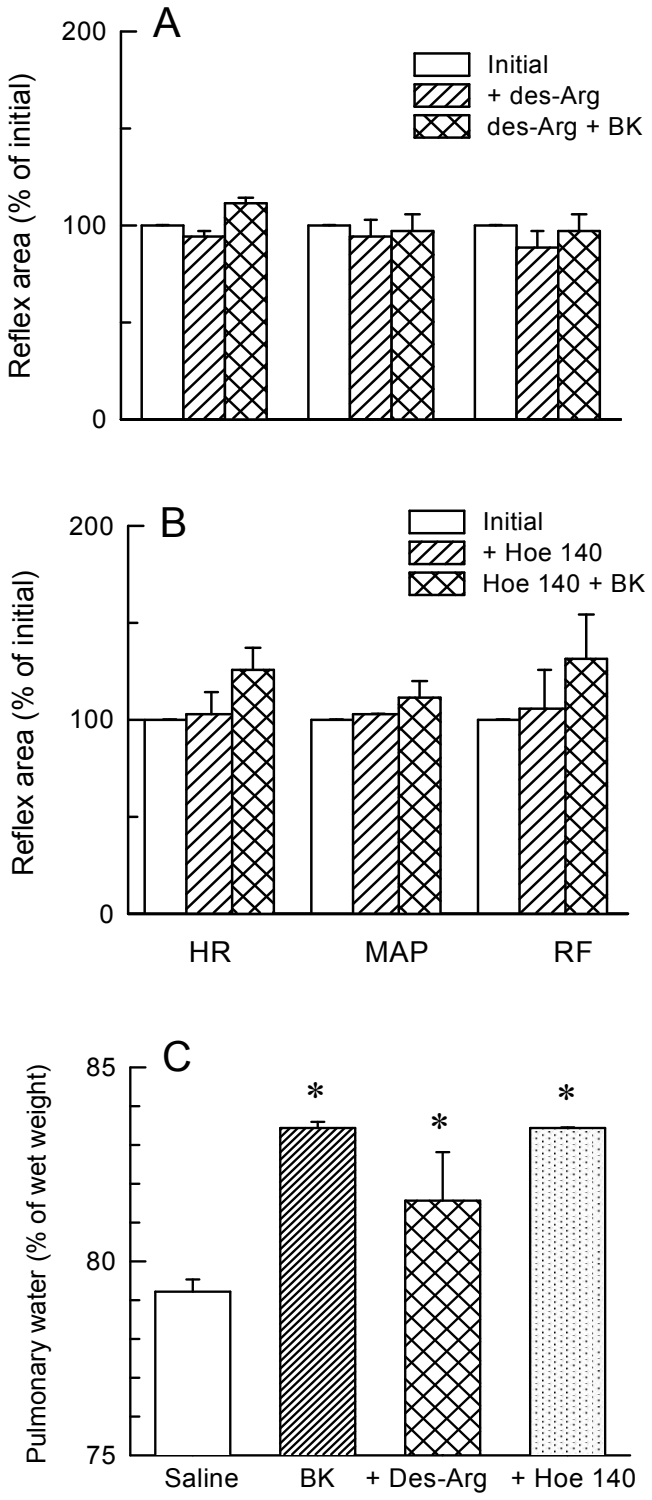

Fig. 4 : BK receptor antagonists attenuated the BK-induced augmentation of PDG reflex. Histograms in A and B show the PDG responses obtained initially (initial), after pretreatment with des-Arg or Hoe 140 and after BK, respectively. In C, the values of pulmonary water content in saline control, BK and antagonist pretreated groups are shown. Each bar represents the mean±SEM from 4-5 experiments. Asterisk (*) represent P<0.05, as compared to control group (Student's *t*–test for unpaired observations). Note: BK induced augmentation observed in Fig. 3 is not seen after B_1 or B_2 receptor antagonist pre-treatment.

study, attenuation of PO-Tx-induced reflex changes and pulmonary oedema by Hoe 140, confirms the involvement of B_2 receptors for the action of PO-Tx similar to crude MBT venom, as reported elsewhere (21). The involvement of B_1 receptors for PO-Tx induced augmentation was not tested in the present study since these receptors are not present constitutively as mentioned above (25). Further, the non-involvement of B_1 receptors has been reported with crude MBT venom (21).

Increased BK levels have been reported after scorpion envenomation (12, 17). BK-induced augmentation of PDG reflex in the present study supports the kinin involvement for PO-Tx induced augmentation. BK also increased the pulmonary water content as seen with PO-Tx in the present study. These results establish the role of kinins in increasing the capillary permeability, and thereby leading to pulmonary oedema after PO-Tx/BK administration. However, the BK-induced reflex augmentation was sensitive to both B_1 and B_2 receptor antagonists, but not the pulmonary oedema (Fig. 4). Whereas, the PO-Tx-induced reflex augmentation as well as pulmonary oedema were sensitive to B_2 receptor antagonist (Fig. 2). Thus PO-Tx induced reflex augmentation appears to be pulmonary oedema dependent, while BK-induced reflex augmentation is independent of pulmonary oedema. The discrepancy in BK-induced reflex augmentation and pulmonary oedema needs further investigation.

The results of the present study hence demonstrate that the action of PO-Tx, a constituent of *Mesobuthus tamulus* venom, involves B_2 kinin receptors. Further, PO-Tx mimics the action of BK resulting in the augmentation of cardiopulmonary reflexes and development of pulmonary oedema. These results imply that PO-Tx increases kinin levels to produce the reflex augmentation and pulmonary oedema.

Acknowledgement

The study is supported by the grants from Indian Council of Medical Research (ICMR), New Delhi. Aparna Akella is a recipient of ICMR Senior Research Fellowship (SRF).

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