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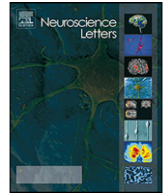

Involvement of phospholipase A_2 pathway for the Indian red scorpion venom-induced augmentation of cardiopulmonary reflexes elicited by phenyldiguanide

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ABSTRACT

The present study was conducted to examine the role of phospholipase A_2 and prostaglandins in Indian red scorpion (*Mesobuthus tamulus*; MBT) venom-induced augmentation of cardiopulmonary reflexes elicited by phenyldiguanide (PDG). Trachea, femoral artery and jugular vein were cannulated in urethane anesthetized adult albino rats. The effect of jugular venous injection of PDG on ECG, BP and respiratory activity were recorded. Injection of PDG (10 μg/kg) evoked tachypnea/apnea, bradycardia and hypotension lasting for 60 s. After injecting MBT venom (100 μg/kg) for 30 min, the PDG evoked reflex responses were augmented by two times and increased the pulmonary water content in envenomed animals, significantly. The venom-induced augmentation of PDG reflex and the increase in pulmonary water content were blocked in animals pretreated with B_2 kinin receptor antagonist (Hoe 140; 2.32 μg/kg). These responses induced by venom were also blocked by a phospholipase A_2 antagonist ($PACOCF_3$; 1 mg/kg) and a prostaglandin synthase inhibitor (indomethacin; 10 mg/kg). The observations indicate that the venom-induced responses (augmentation of PDG reflex response and increased pulmonary water content) involve PLA_2–prostaglandin pathway that is triggered by B_2 kinin receptors to sensitize the receptors located on the vagal C-fibres.

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Juxta-pulmonary capillary receptors (J-receptors) described by Paintal [14] are located on vagal terminals in the interstitium of the lungs. These receptors are excited by pulmonary oedema, pulmonary congestion and chemicals such as serotonin, histamine, phenyldiguanide (PDG), veratridine, etc. [9,14,15]. These chemicals also excite the coronary receptors to evoke similar response pattern known as Bezold–Jarisch reflex. Activation of either of the receptors by PDG, evokes a reflex response (apnea, bradycardia and hypotension) in experimental animals by involving high threshold vagal fibres originating from the heart and lungs [9,15]. Indian red scorpion (*Mesobuthus tamulus*; MBT) venom is shown to augment the PDG-induced reflex responses [2,21] and aprotinin, a kinin synthase inhibitor, blocked it [2,7]. The augmentation of PDG reflex response is shown to be due to the generation of pulmonary oedema produced by venom [2,7]. In addition, the venom-induced augmentation of the reflexes is associated with increased vagal discharges evoked by PDG through a kinin-dependent mechanism [3].

Kinins exert their effects through B_1 and B_2 subtypes of receptors. The B_1 receptors are inducible while B_2 receptors are constitutive. It is shown that the activation of kinin receptors triggers the intracellular signaling pathways that involve phopholipase A_2 and others, producing the physiological and pathophysiological effects [4,20]. Phopholipase A_2 is responsible for the formation of arachidonic acid (from membrane phospholipids), a substrate for the synthesis of prostaglandins. In reports elsewhere, B_2 receptor activation increased the arachidonic acid release [10] and also increased the prostaglandins [5]. Thus, it is expected that venom may also involve similar PLA_2-dependent mechanisms to produce the augmentation of the PDG reflex response. In order to test the above hypothesis, the present study was conducted to look into the subsequent downstream intracellular signaling mechanisms of kinins involving membrane phospholipids for the sensitization of receptors located on vagal C-fibres.

Female albino rats of Charles Foster strain weighing 200–220 g were anesthetized with urethane (1.5 g/kg, i.p.). Trachea, jugular vein and femoral artery were cannulated as mentioned in our previous reports [2,7]. The tracheal cannulation was done to keep the respiratory tract patent. Right jugular venous cannulation was used for administering the saline/antagonists/venom. The femoral artery cannulation was utilised for recording the blood pressure

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by connecting it to Statham transducer. Respiratory movements were recorded using the force displacement transducer by securing it over the xiphisternum by a thread. Electrocardiographic (ECG) potentials were recorded by connecting needle electrodes in Limb lead II configuration. The animals were allowed to stabilize for 30 min before making any recordings on the chart recorder.

Crude *M. tamulus* (MBT) venom was procured from Haffkine Institute, Mumbai, India. Phenyldiguanide (PDG) was obtained from Koch Light Laboratories, Bucks, U.K., Hoe 140 (H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH trifuoroacetate), $PACOCF_3$ (palmitoyl tri flouro acetate) and indomethacin were purchased from Sigma Chemical Company, St. Louis, MO, USA. The stock solution (1 mg/ml) of all the drugs was prepared in distilled water except indomethacin. Indomethacin (20 mg/ml) was prepared freshly every time in 100% ethanol.

The effect of PDG reflex response before and after venom was demonstrated in the first group of rats (venom only group). In this group, PDG reflex response was obtained initially, 20 min after saline and 30 min after the injection of venom (100 μg/kg). In the second group, the effect of various antagonists (Hoe 140/$PACOCF_3$/indomethacin/ethanol-a vehicle for indomethacin) on the responses induced by venom were examined. In this group, the PDG response was obtained initially, 20 min after exposure to antagonist/vehicle and 30 min after injection of venom (100 μg/kg). In both the groups, the volumes of intravenous injections of PDG or antagonists or venom used were 0.1 ml. At the end, the lungs were dissected out, blotted gently and weighed (wet weight). The tissue was then chopped into smaller pieces and kept for drying in an oven (at 90 °C) to a constant weight (dry weight) to determine the water content as described earlier [7].

The time–response area of the PDG reflex was computed as the responses lasted for >60 s. The area was calculated by plotting a graph of each responses at every 5 s up to 60 s as mentioned before [7,19,21]. The response area after saline or after various antagonists or venom was normalised to the initial PDG response area and were pooled to obtain the mean ± S.E.M. The differences in pretreated groups were compared with the venom only group using Student's *t*-test for paired or unpaired observations. A *P*-value <0.05 was considered significant.

The original tracings of reflex responses evoked by PDG before and after exposure to venom in an experiment are shown in Fig. 1a. Jugular venous injection of PDG (10 μg/kg) produced tachypnea followed by bradyapnea, bradycardia and hypotension lasting for >60 s. The time–response area of the PDG reflex responses as normalised to the initial PDG response are presented in histogram (Fig. 1b). Exposure to venom (100 μg/kg) for 30 min augmented

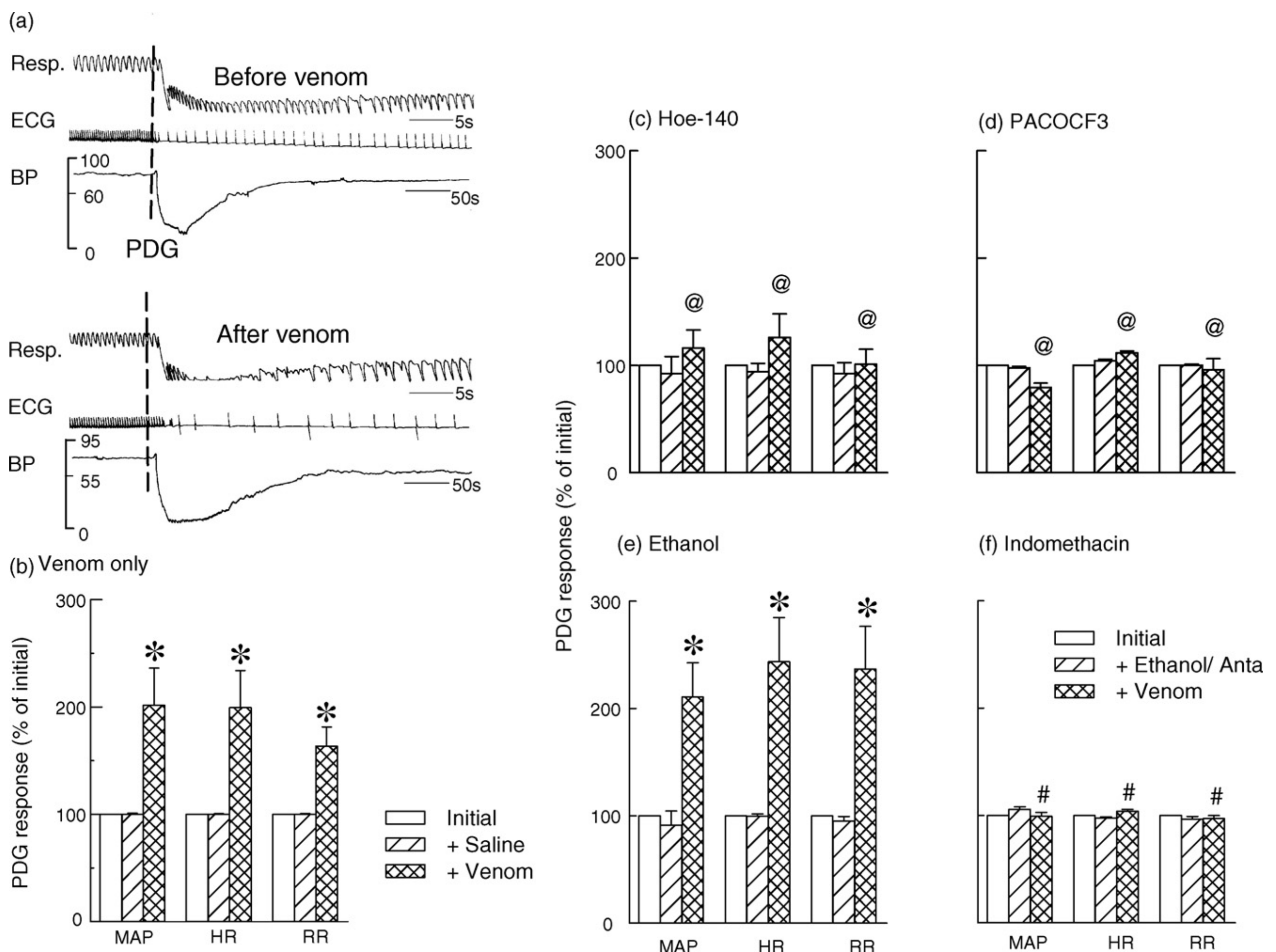

Fig. 1. Effect of various antagonists on the venom-induced augmentation of PDG reflex responses. In (a), the original tracings of an experiment showing the PDG reflex response before and after MBT venom (100 μg/kg) on respiration (Resp.), ECG and blood pressure (BP) are given to demonstrate the temporal dispersion of the responses. Dashed lines indicate the point of injection of PDG (10 μg/kg). The time calibrations for Resp. and ECG are same. The pooled data of time–response area of PDG reflex are presented as mean ± S.E.M. in graphs b–f. The PDG response was elicited initially (initial), 20 min after injecting saline (+saline) or antagonist (Anta) or ethanol (+ethanol) and 30 min after venom (+venom) in these groups as indicated. *Indicates significant difference from the initial (*P* < 0.05, Student's *t*-test for paired observations); @indicates *P* < 0.05, Student's *t*-test for unpaired observations as compared to venom only response; #indicates *P* < 0.05, as compared to ethanol.

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