Reflex hypertensive response induced by capsaicin involves endothelin-dependent mechanisms

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Abstract
Capsaicin, a nociceptive agent produces triphasic pressure response in rats. The mechanisms underlying capsaicin-induced pressure responses are not clear. Therefore, the present study was undertaken to determine the mechanisms involved in capsaicin – induced pressure responses. The trachea, jugular vein and femoral artery were cannulated in anaesthetized rats. Capsaicin (10 µg/kg; i.v) - induced reflex changes in the blood pressure, respiratory excursions and ECG were recorded before/after vagotomy in the absence/presence of antagonists. Capsaicin produced the triphasic pressure response characterized by immediate fall, recovery (intermediate phase) and delayed progressive fall. After vagotomy, the immediate hypotension was abolished and the intermediate pressure response was potentiated as a hypertensive response while the delayed hypotensive response persisted. The time-matched heart rate changes (bradycardia) and respiratory changes (tachypnea in delayed phase) were abolished after vagotomy. Pretreatment with endothelin receptor antagonist (bosentan; 10 mg/kg) blocked the capsiaicn-induced intermediate hypertensive response in vagotomised animals but not the delayed hypotension. Pretreatment with nitric oxide synthase (NOS) inhibitor (L-NAME; 30 µg/kg), prostaglandin synthase inhibitor (indomethacin; 10 mg/kg) and kinin synthase inhibitor (aprotinin; 6000 KIU) did not block the delayed hypotensive response. These results demonstrate that capsaicin-induced intermediate hypertensive response involves endothelin-dependent mechanisms and the delayed hypotensive response is independent of nitrergic, prostaglandinergic or kininergic mechanisms.

Introduction
Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a nociceptive substance present in Capsicum annuum. In mammals, it produces burning sensation in skin and mucous membranes upon contact. Capsaicin is an agonist for the transient receptor potential vanilloid receptors-1 (TRPV1). TRPV1 are non-selective cation channels expressed on various neuronal and non-neuronal tissues (1, 2). They are sensitive to temperature, protons, cations and noxious stimuli. Accidental consumption of capsaicin has been shown to produce hypertensive crisis (3). In addition, stimulation of TRPV1 on visceral or somatic afferents produces changes in blood pressure (4-7). Intravenous injection of capsaicin produced cardio-respiratory reflexes in rats (7). Recently, capsaicin-induced...
pressure responses in rats have been shown to be typically triphasic in nature (5-7). It is characterized by hypotension in the immediate phase followed recovery in the intermediate phase and again hypotension in the delayed phase. The capsaicin-induced immediate response has been shown to be mediated through the vagal pathway (6, 7). However, vagotomy has been shown to potentiate the intermediate phase of capsaicin-induced pressure response as a hypertensive response (6, 7). In a study elsewhere, non-involvement of adrenergic and angiotensinergic mechanisms for the capsaicin-induced intermediate hypertensive response has been shown (6, 7). In the absence of adrenergic/angiotensinergic mechanisms the involvement of vasoconstrictors like endothelin (produced by the vascular endothelial cells) is expected. Endothelin acts via two types of receptors called ET-A and ET-B. Activation of ET-A receptors produces vasoconstriction while ET-B receptors induces vasodilation (8). Endothelin-1 acting via ET-B receptors stimulates the release of vasodilators like nitric oxide (NO) and prostacyclins (1, 8). Hence, it is hypothesized that the capsaicin-induced intermediate pressure response is mediated by endothelin-1 acting via ET-A receptors and the capsaicin-induced delayed hypotensive response occurs due to ET-B receptor mediated mechanisms. The present study was therefore carried out to determine the role of endothelin in mediating capsaicin-induced intermediate hypertensive and delayed hypotensive responses.

Material and Methods

Animals

Experiments were performed after obtaining the approval from the Institutional Ethical Clearance Committee for conducting animal experiments. Adult female rats of Charles Foster strain of 176±14 g were used. The animals were housed in a temperature, humidity and light (12 h: 12 h light dark period) controlled room with ad libitum food and water.

Dissection and recording

The methods for dissection and recording of cardio-respiratory parameters were as described earlier (9). Briefly, 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 as assessed by the corneal and limb withdrawal reflexes. Tracheal cannulation was used to keep the respiratory tract patent; jugular venous cannulation for capsaicin/antagonist administration; and femoral artery cannulation for recording blood pressure via Statham transducer. Electrocardiographic (ECG) 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 the xiphisternum to a force-displacement transducer. All the recordings were taken on a computerized chart recorder (ADInstruments, Australia).

Drugs and solutions

Capsaicin, aprotinin, L-NAME and indomethacin were obtained from Sigma Chemical Company St. Louis, MO, USA. Bosentan was procured from Cipla, Goa, India. Stock solution of capsaicin (1 mg/ml), bosentan (20 mg/ml) and indomethacin (10 mg/ml) were prepared in ethanol. Stock solutions of L-NAME and aprotinin (1 mg/ml) were prepared in distilled water. The stock solutions were diluted with normal saline at the time of administration. The volume of the injections was kept at 0.1 ml.

Experimental protocol

The animals were divided into three groups. In group I, after obtaining the initial recordings (respiration, ECG, and blood pressure), capsaicin (10 µg/kg) was injected intravenously. The blood pressure, respiratory excursions and ECG were recorded for 1 min. Subsequently, bilateral vagotomy was performed and 10 min later capsaicin (10 µg/kg) response was obtained again.

In group II, the capsaicin-induced responses were obtained as described in group I. Subsequently, animal was pretreated with bosentan (ET-A and ET-B receptor antagonist; 10 mg/kg; n = 4); L-NAME (NOS inhibitor; 30 µg/kg; n = 3) or indomethacin (prostaglandin synthase inhibitor; 10 mg/kg; n = 3) and 15 min later capsaicin (10 µg/kg) reflex response
was recorded again. The doses of antagonists were selected from the earlier reports (10-12).

In group III, after obtaining the initial capsaicin response, the animal was pretreated with aprotinin (kinin synthase inhibitor; 6000 KIU; n = 3) and 15 min later capsaicin-induced response was obtained again. The dose of aprotinin was taken from an earlier report (12).

Statistical analysis

Peak changes in mean arterial pressure (MAP; mmHg) from the initial at different phases after capsaicin were calculated. The time-matched changes in respiratory rate (RR) and heart rate (HR) at immediate (0-10 sec), intermediate (10-20 sec) and delayed (45-60 sec) phases of blood pressure response were calculated. The responses were normalized to the respective initial values (before capsaicin administration). The data were pooled to obtain mean±SEM. One way ANOVA followed by Student Newman Keul’s test was used to compare the significant difference between the values before vagotomy, after vagotomy and after pretreatment with antagonists. In addition, Student’s t test was used to compare the paired responses before and after vagotomy. A P<0.05 was considered significant.

Results

Capsaicin produced triphasic pressure response

As reported elsewhere (6), bolus injection of capsaicin (10 µg/kg) produced triphasic pressure responses that can be categorized as immediate hypotension, intermediate recovery and a delayed hypotension, respectively (Fig. 1A and D). After vagotomy, the capsaicin-induced immediate hypotensive response was abolished, intermediate recovery was potentiated as hypertensive response and delayed hypotensive response persisted (Fig. 1A and B).

ET-A and ET-B receptor antagonist (bosentan) blocked the capsaicin-induced intermediate hypertensive response but not the delayed hypotensive response.

Bosentan per se did not alter the basal MAP, RR and HR values seen after vagotomy (Table I). Pretreatment with bosentan, blocked the potentiation of the intermediate pressure response however, the delayed hypotension persisted (Fig. 1C and D).

L-NAME, indomethacin and aprotinin did not block the capsaicin-induced delayed hypotensive response.

L-NAME (NOS inhibitor)/indomethacin (prostaglandin synthase inhibitor) per se did not alter the basal MAP, RR and HR values seen after vagotomy (Table I). Pretreatment with L-NAME/indomethacin, did not block the delayed hypotensive response (Fig. 2A and B).

Aprotinin (kinin synthase inhibitor) per se did not alter the basal MAP, RR and HR values (Table I). The capsaicin-induced responses in the immediate and intermediate phases after pretreatment with aprotinin were similar to the initial capsaicin-responses. Further, aprotinin pretreatment did not block the delayed hypotensive response (Fig. 2C).

Effect of various antagonists on the capsaicin-induced changes in HR and RR at different time phases

The time-matched heart rate changes after capsaicin administration manifested as bradycardia at all phases and they were abolished after vagotomy (Table II). Antagonist pretreatment did not alter the
TABLE II: Capsaicin-induced HR and RR changes at immediate, intermediate and delayed phases in control, vagotomized and antagonist treated animals are given below. The mean±SEM values are from 3-4 different experiments. An asterisk (*) indicates P<0.05, One way ANOVA followed by Students Newman Keul’s test. The corresponding basal values of HR and RR in different groups are given in Table I. + Antagonist indicates bilateral vagotomy + antagonist pretreatment.

<table>
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<tr>
<th></th>
<th>Immediate (%)</th>
<th>Intermediate (%)</th>
<th>Delayed (%)</th>
<th>Immediate (%)</th>
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<td>108±15.3</td>
<td>86.9±9.9*</td>
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<tr>
<td>+ Bosentan</td>
<td>99.6±1.0*</td>
<td>100.5±0.7*</td>
<td>100.9±0.7*</td>
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<td>Capsaicin</td>
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Fig. 1: Bosentan (endothelin receptor antagonist) blocked the capsaicin-induced intermediate hypertensive response. The original tracings of an experiment showing the capsaicin (10 µg/kg)-induced changes in blood pressure (BP); respiration (Resp); and heart rate (ECG), before vagotomy (A), after vagotomy (B) and after bosentan in an experiment (C). In B, an arrow depicts the intermediate hypertensive response. Vertical dashed line indicates the point of injection of capsaicin (10 µg/kg). Horizontal dashed line within BP recording shows the initial mean arterial pressure. The bar diagram in D shows the mean±SEM values (n = 4) of MAP at immediate, intermediate and delayed phases before vagotomy (Caps), after vagotomy (Vag X) and after bosentan. An asterisk (*) indicates P<0.05, from the values before vagotomy at each phase (one way ANOVA followed by Student- Newman Keul’s test); @ indicates P<0.05 from values after vagotomy (Student’s t-test for paired observations).
Fig. 2: Capsaicin-induced delayed hypotensive response is not blocked after pretreatment with L-NAME, indomethacin or aprotinin. The bar diagrams show means±SEM values (n = 3) of capsaicin-induced changes in MAP at immediate, intermediate and delayed phases before and after vagotomy and after pretreatment with L-NAME (A), indomethacin (B) or aprotinin (C). Caps, initial capsaicin response before vagotomy; VagX, capsaicin response after vagotomy; and Indo, capsaicin response after indomethacin pretreatment. An asterisk (*) indicates P < 0.05 from the values before vagotomy at each phase (one way ANOVA followed by Student-Newman Keul’s test).
HR changes any further. In the aprotinin pretreated group (where vagotomy was not performed), the HR changes in different time phases remained similar to the initial capsaicin-responses (Table II).

The respiratory changes exhibited immediate bradypnea and delayed tachypnea (Table II). The delayed tachypnea was abolished after vagotomy while immediate bradypnea persisted (Table II). Aprotinin pretreatment did not alter the RR changes any further. In the aprotinin pretreated group (where vagotomy was not performed), the RR changes in different time phases remained similar to the initial capsaicin-responses (Table II).

Discussion

The present observations demonstrate that endothelin-dependent mechanisms are responsible for the capsaicin-induced intermediate hypertensive response. However, the capsaicin-induced delayed hypotensive response does not involve NO, PGs or kinins.

Capsaicin mediates its actions through TRPV1 receptors. TRPV1 receptors are a type of nociceptors present on the vagal afferents innervating cardiovascular and renal tissues (2). Capsaicin stimulates the TRPV1 receptors present on pulmonary vagal C fibers to produce the cardiorespiratory reflexes (4-6, 12, 13). These reflexes manifested as bradycardia, bradypnea and triphasic pressure response as seen earlier (6, 7). In this study also, the triphasic pressure response is characterized by immediate hypotension, intermediate recovery and delayed hypotension as seen earlier (6, 7).

In the present study and elsewhere, the capsaicin-induced immediate hypotensive response was abolished after vagotomy implicating the involvement of vagally mediated mechanisms (6, 7). However, vagotomy potentiated the intermediate pressure response and manifested as a hypertensive response. Hypertension can be due to adrenergic mechanisms, action of vasoconstrictors like angiotensin, endothelin or depletion of vasodilator substances like CGRP (2, 3). In earlier study, the non-involvement of adrenergic, angiotensinergic and calcium channel – dependent mechanism for the capsaicin-induced hypertensive response has been reported (6, 7). Our results with bosentan in the present study demonstrate for the involvement of endothelin-1 receptor for mediating the capsaicin-induced hypertensive response (Fig. 1).

Bosentan (antagonist for both ET-A and ET-B receptors) blocked the intermediate hypertensive response but not the delayed hypotensive response (Fig. 1C and D). Bosentan is an antagonist for both ET-A and ET-B receptors. Activation of ET-A receptors produce hypertension while ET-B receptors produces hypotension (8). The blockade of capsaicin-induced hypertensive response by bosentan demonstrates the involvement of ET-A receptors. However, persistence of capsaicin-induced delayed hypotensive response even after bosentan demonstrates the non-involvement of ET-B receptors. Hypotension can also be due to vasodilators like NO, PGs and kinins. Our results with NOS inhibitor (L-NAME) and prostaglandin synthase inhibitor (indomethacin) implicate for the non-involvement of nitrergic and prostaglandinergic mechanisms for the capsaicin-induced delayed hypotensive responses (Fig. 2A and B).

Kinins are another group of potent vasodilators, implicated for mediating capsaicin-induced responses (4). In the absence of nitrergic and prostaglandinergic mechanisms, we examined the involvement of kinins for the capsaicin-induced delayed hypotension. However, our results with aprotinin implicate for the non-involvement of kininergic mechanisms for the capsaicin-induced delayed hypotensive response (Fig. 2C). In the absence of involvement of ET-B receptors, NO, PG or kininergic mechanisms, the capsaicin-induced release of vasodilators like CGRP and substance P remains a possibility (2, 14-16).

Conclusion

The capsaicin-induced intermediate hypertensive response involves endothelin-1 acting via ET-A receptors. However, the capsaicin-induced delayed hypotensive response is independent of ET-B receptors,
nitrergic, prostaglandinergic or kininergic mechanisms.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References