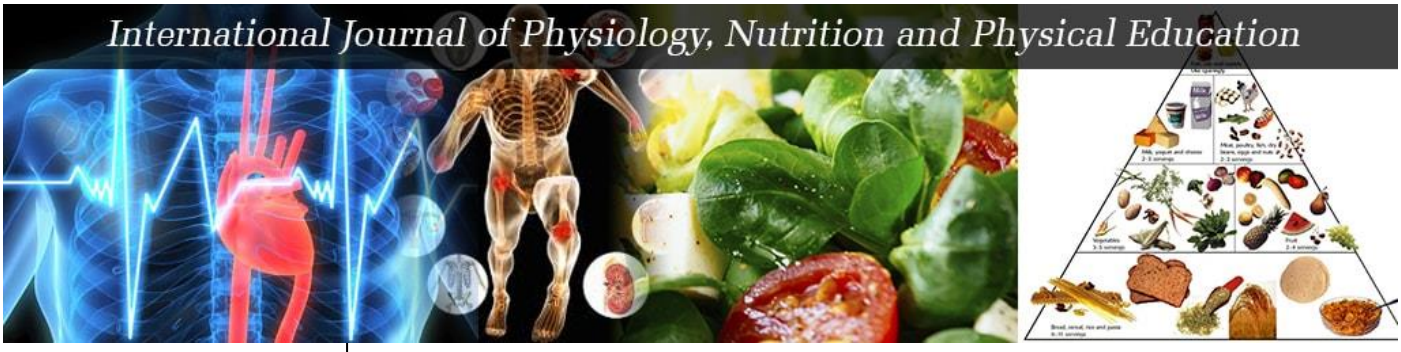

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Capsaicin-induced cardio-respiratory responses in vagotomized animals are not mediated through adrenergic or angiotensinergic system

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Abstract
Capsaicin produces complex Triphasic cardio-respiratory responses characterized by immediate hypotension associated with apnea, intermediate recovery with bradypnea and delayed hypotension with tachypnea. However, the mechanisms underlying this complex cardio-respiratory responses are not clear. Therefore, this study was undertaken to delineate the mechanisms involved in capsaicin-induced respiratory response in connection to pressure responses. Tracheal, jugular venous and femoral artery cannulations were performed in urethane anaesthetized adult rats. Blood pressure, and respiratory excursions were recorded. Jugular venous injection of capsaicin (10µg/kg) produced Triphasic pressure response exhibiting immediate hypotension, intermediate recovery and delayed hypotension. Time-matched respiratory changes showed apnea, bradypnea and tachypnea, respectively. After vagotomy, immediate hypotension was abolished; the intermediate recovery in presser response was augmented as hypertensive response; and the delayed hypotension persisted. In these animals, immediate apnea was attenuated, intermediate apnea/bradypnea persisted and delayed tachypnea was abolished after vagotomy. Antagonists of α_1-Adrenoceptor (proposing, 0.5mg/kg) or AT_1 receptor (losartan, 10mg/kg) did not block the capsaicin-induced intermediate hypertensive response as well as apnea/bradypnea in vagotomized animals. The present observations indicate that capsaicin-induced intermediate hypertensive response in association with apnea/bradypnea in vagotomized animals is not mediated through adrenergic and angiotensinergic mechanisms. The direct modulation of central respiratory area may be a possibility.

Keywords: Capsaicin, proposing, losartan, adrenoceptor antagonist, angiotensin 1 receptor antagonist
Abbreviations: AT_1 – angiotensin II type 1, RF - respiratory frequency, MAP - mean arterial pressure

Introduction
Capsaicin is a nociceptive agonist present in *Capsicum annuum* and it is a potent stimulant of C fibres. Intravenous injection of capsaicin produces reflex changes in cardio-respiratory parameters manifesting as apnea, bradycardia and hypotension in anaesthetized rats [9, 10]. Similar responses are also reported in cats, dogs and monkeys after capsaicin injection (I.V.) [4, 11, 12, 16]. Afferents in mediating these responses are shown to be present in vagus as bilateral vagotomy abolished these cardio-respiratory alterations in rats, cats and dogs but not in monkeys [4, 10, 11, 12, 16]. In a study elsewhere Triphasic pressure response was observed instead of only hypotension after intravenous administration of capsaicin in rats but the Triphasic response was partially abolished after bilateral vagotomy [3, 5, 14]. Further, capsaicin-induced Triphasic response persisted even in spinal animals and also did not involve cholinergic and adrenergic efferent [5]. In our previous study, we have observed Triphasic pressure response such as immediate hypotension, intermediate hypertension and delayed hypotension associated with apneic, bradypnic and tachypnic respiratory responses respectively. Bilateral vagotomy abolished the immediate hypotensive response however the associated apnea persisted partially. Further, the intermediate hypertensive response was augmented and bradypnic response persisted and delayed hypotension was not altered but associated tachypnea was completely abolished after vagotomy (6). Thus, the nature and mechanisms underlying the respiratory responses induced by capsaicin are still not clear. Therefore, present study was undertaken to understand whether the respiratory response was secondary to hypertensive response as induced by intravenous capsaicin in anesthetized rats and the role of vagus,

adrenergic or angiotensinergic mechanisms in mediating these responses was evaluated.

Material and methods

Animals

Experiments were performed according to the guidelines of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India for conducting animal experiments. Adult female rats of Charles Foster strain weighing 196±32g were used. The animals were housed in a temperature, humidity and light (12h: 12h 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 (6, 10). Briefly, animals were anaesthetized with urethane (1.5g/kg I.P). An additional dose of urethane (0.1-0.15g/kg I.P) was injected whenever required as assessed by the corneal and withdrawal reflexes to noxius stimuli. Trachea, jugular vein and femoral artery were annulated. 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 Stathum transducer. 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.

Drugs and solutions

Capsaicin, proposing (α_1-adrenoceptor antagonist), and losartan (AT_1 receptor antagonist) were obtained from Sigma Chemical Company St. Louis, MO, USA. Stock solution of capsaicin (1mg/ml) was prepared in ethanol. Stock solution of other drugs was made in distilled water and diluted with normal saline at the time of administration. The volume of the injections was kept at 0.1ml.

Experimental protocols

The animals were divided broadly into 2 groups: In group-I (N=10), after obtaining the baseline recordings (respiration and blood pressure) capsaicin (10µg/kg) was administered intravenously. The arterial pressure and respiration were recorded for 1 min. Subsequently, bilateral vagotomy was performed and 10 min later capsaicin (10µg/kg) response was obtained as before.

In group-II (N=12), capsaicin (10µg/kg)-induced reflex response was recorded initially and after bilateral vagotomy as described in group I. Subsequently, proposing (0.5mg/kg) and losartan (10mg/kg) was injected and 15 min later capsaicin (10µg/kg) reflex response was recorded. The dosage of both antagonists was selected from the earlier reports [2, 5, 13].

At the end of experiments, the animals were killed by administration of over dose of anesthesia.

Statistical analysis

Peak changes in mean arterial pressure (mmHg) from baseline at different phases after capsaicin were computed and were expressed as % of initial. In case of respiration, time-matched respiratory frequency (RF) at immediate, intermediate and delayed phases of blood pressure response were computed after capsaicin administration. The responses were normalized to the respective baseline values (before capsaicin administration). The data were pooled to obtain mean ± SEM. Student's *t*-test for paired observations was performed to compare changes after vagotomy or after pretreatment with antagonists in vagotomized animals. A P <0.05 was considered significant.

Results

Capsaicin produced Triphasic cardio-respiratory response

In this series of experiments (N=10), injection of capsaicin (10µg/kg) produced changes in MAP in a time-dependent manner. These changes are seen as immediate fall (by 50%) after a latency of 1.4±0.3s and lasted for 5-6s. Subsequently, the fall in pressure was recovered and peaked slightly above the initial level (107% of initial; Fig. 1). The latency and duration of this response was 6.1±0.4s and 10 s, respectively. Thereafter, there was a delayed fall in pressure (by 30%) with a latency of 17.7±1.4s and persisted for longer time. Thus, the MAP response can be categorized as immediate hypotensive, intermediate recovery and a delayed hypotensive response. The time-matched respiratory responses to the pressure responses manifested as immediate apnea, intermediate bradypnea and delayed tachypnea, respectively (Fig. 1).

Capsaicin-induced intermediate presser response was augmented after vagotomy but apnea/bradypnea persisted

The initial values of MAP and RF before vagotomy were 96 mmHg, and 68 breaths/min respectively. After bilateral vagotomy, RF was decreased by 52% ($P < 0.05$, Student's *t* test for paired observations) while there was no significant alteration in MAP. In vagotomized animals, the intermediate presser response was augmented (43% increase; $P < 0.05$, Student's *t* test for paired observations). In these animals, capsaicin-induced immediate hypotension was abolished while RF manifested as bradypnea as compared to apnea seen in pre-vagotomy experiments ($P < 0.05$, Student's *t* test for paired observations). The delayed hypotensive response persisted even after vagotomy while tachypnea was abolished (Fig 1).

Since, intermediate pressor response was augmented after vagotomy, further experiments were performed to delineate the relationship of pressor response with apneic/bradypneic response.

α_1-Adrenoceptor antagonists neither blocked the augmented pressor response nor apnea/bradypnea in vagotomized animals

The results of proposing treated group are presented in Fig 2. In this group, capsaicin-induced Triphasic pressure response before and after vagotomy were similar to the earlier group (Fig 1). In vagotomized animals proposing (α_1-adrenoceptor antagonist; 0.5mg/kg) *per se* decreased the resting MAP significantly (Table 1) but RF was not different from the pre-proposing values. After proposing pretreatment the intermediate hypertensive response as well as the apnea/ bradypnia produced by capsaicin was not reversed to initial level (Fig 2).

Table 1: Mean arterial pressure (MAP) and respiratory frequency (RF) in animals before, after vagotomy and after respective antagonist. The mean ± S.E.M. values were obtained from 3-4 experiments in each group

Group	Before	After vagotomy	After antagonist
Proposing group			
MAP (mmHg)	99.7 ± 2.8	101.3 ± 3.2	66.3 ± 3.8 @
RF (per min)	66 ± 3.5	30 ± 3.5*	42 ± 3.5
Losartan group			
MAP (mmHg)	100.5 ± 10.9	90.0 ± 10.8	60.2 ± 9.9@
RF (per min)	70 ± 3.8	39 ± 7.5*	43 ± 5.8

* P < 0.05 as compared with before values (Student's t test for paired observations)
@ P < 0.05 as compared with after vagotomy values (Student's t test for paired observations)

AT_1 receptor antagonist failed to block the augmented pressor response as well as the apnea/bradypnea in vagotomized animals

The results of this group are presented in Fig 3. In this group, capsaicin-induced Triphasic pressure response before and after vagotomy were similar to the earlier group (Fig 1). In vagotomized animals losartan (AT_1 receptor antagonist; 10mg/kg) *per se* decreased the resting MAP significantly (Table 1) but RF was not different from the pre-losartan values. After losartan pretreatment the intermediate hypertensive response produced by capsaicin was not blocked (Fig 3) and apnea /bradypnea persisted.

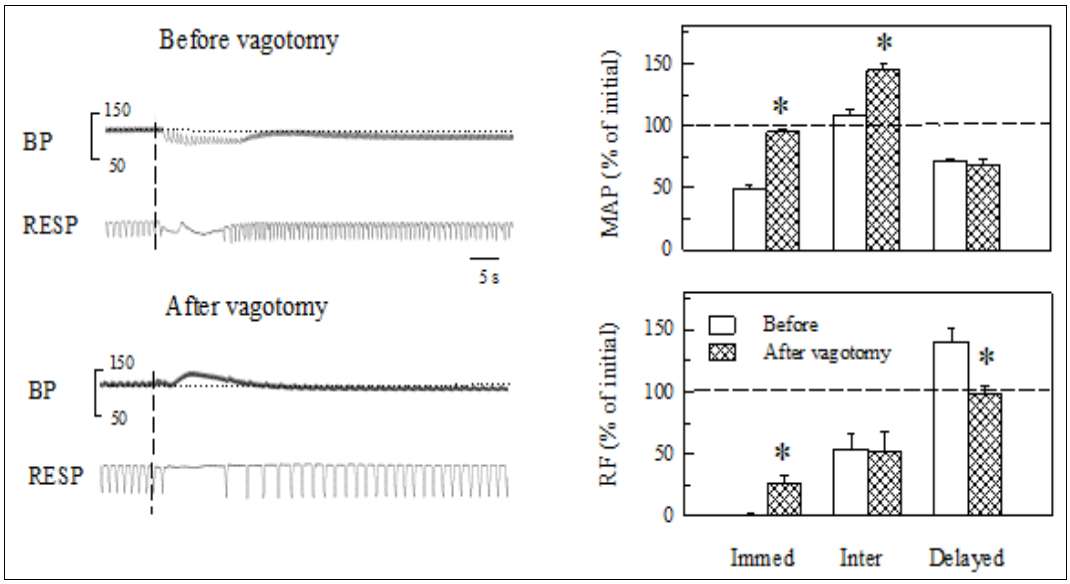
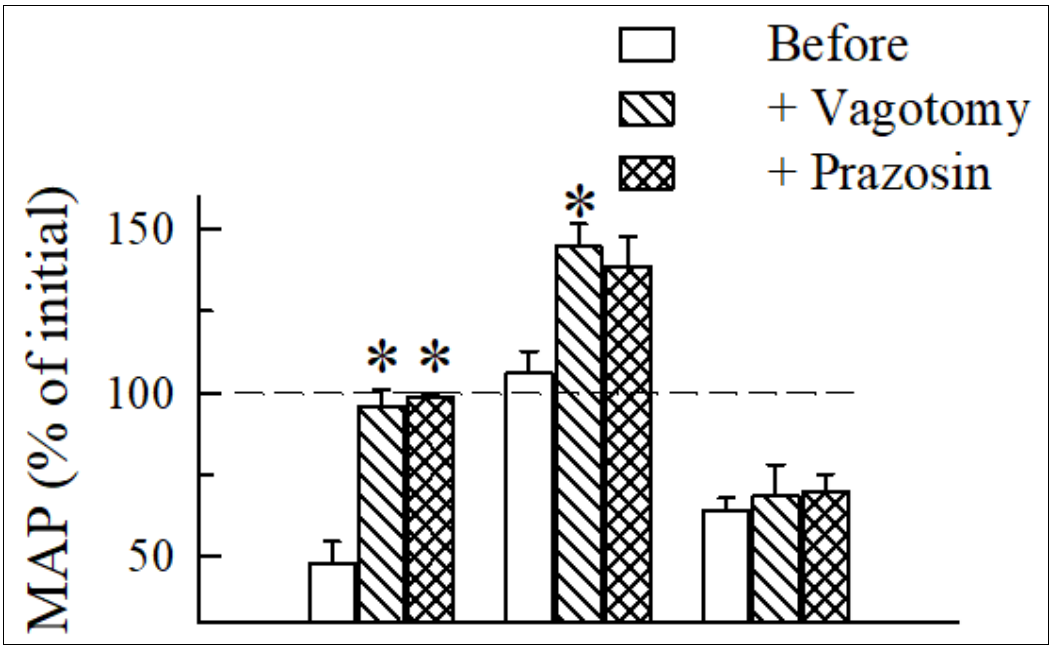


Fig 1: Capsaicin-induced cardio-respiratory responses before and after vagotomy. The original tracings of an experiment showing the capsaicin (10µg/kg)-induced changes in blood pressure (BP) and respiration (Resp) before and after vagotomy are presented in the left panel. Vertical dashed line indicates the point of capsaicin (10µg/kg) administration. Horizontal line (time scale) = 5s. The mean ± SEM values (N=12) of mean arterial pressure (MAP) and respiratory frequency (RF) as % of initial at immediate (Immed), intermediate (Inter) and delayed phases are presented in the bar diagrams. An asterisk (*) indicates significant difference from before values ($P < 0.05$, Student's t test for paired observations)



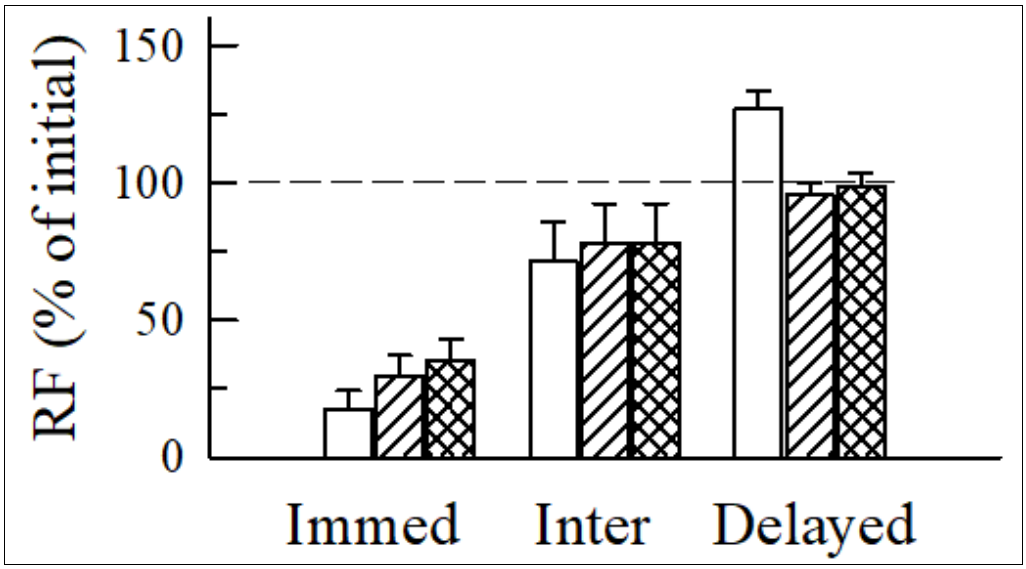


Fig 2: The capsaicin (10µg/kg)-induced intermediate hypertensive response in vagotomized animals was not blocked by α_1 adrenoceptor antagonist. The mean ± SEM values (N=3) of MAP as % of initial at immediate (Immed), intermediate (Inter) and delayed phases are presented in the bar diagrams. Before indicates before vagotomy; + Vagotomy indicates after vagotomy; and + Proposing indicates proposing after vagotomy. An asterisk (*) indicates significant difference from the corresponding before values ($P < 0.05$, Student's t test for paired observations).

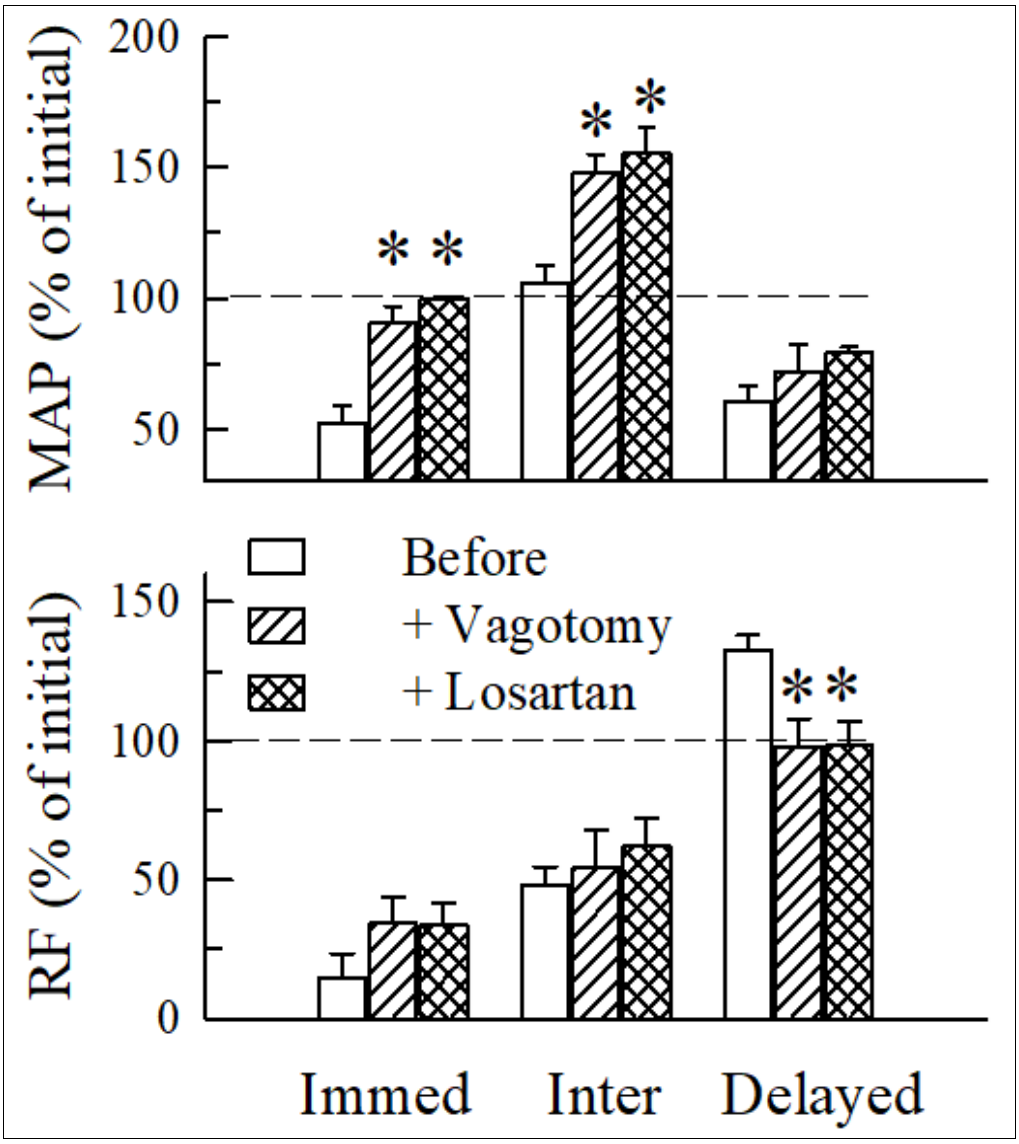


Fig 3: The capsaicin (10µg/kg)-induced intermediate hypertensive response in vagotomized animals was not blocked by AT_1-receptor antagonist. The mean ± SEM values (N=4) of MAP as % of initial at immediate (Immed), intermediate (Inter) and delayed phases are presented in the bar diagrams. Before indicates before vagotomy; + Vagotomy indicates after vagotomy; and + Losartan indicates losartan after vagotomy. An asterisk (*) indicates significant difference from the corresponding before values ($P < 0.05$, Student's t test for paired observations).

Discussion

The present results reveal that jugular venous administration of capsaicin produces Triphasic blood pressure response characterized by fall, recovery and a prolonged fall in association with apnea, bradypnea and tachypnea respectively. Vagotomy abolished the immediate hypotension in association with apneic response and delayed hypotensive response along with the tachypnic responses but not the intermediate pressor response in conjugation with the apneic/bradypnic response. In contrast, the intermediate pressor response was augmented and manifested as hypertensive response after vagotomy. The intermediate hypertensive response and associated apnea/bradypnea after vagotomy can be due to the increased by cardiac activity or due to increased peripheral vascular tone.

In our earlier study, vagotomy completely abolished the capsaicin induced bradycardia response in immediate and intermediate phases whereas pressor response was augmented in intermediate phase. Thus, hypertensive response associated with apnea/bradypnea in intermediate phase due to heart rate changes is quite unlikely.

In the absence of vagal control, hypertension can be due to sympathetic over activity on heart and peripheral vessels. In the absence of cardiac involvement, hypertensive response can be due to the increased peripheral vasoconstriction produced by sympathetic over activity at α_1-adrenoceptors. Our results exclude the involvement of α_1-adrenoceptors as proposing or terazosin (α_1-adrenoceptor antagonists) did not block the hypertensive response and associated apnea/bradypnea in vagotomized animals (Fig 2). Similarly, in a study elsewhere, the hypertensive response to capsaicin was not attenuated in spinalized rats [5]. They further demonstrated that phentolamine or propranolol failed to block the hypertensive response with capsaicin [5]. Thus, these findings support our observations for the non-involvement of α_1 or β adrenoceptors in pressor induced apnea/bradypnea in intermediate phase.

In the absence of sympathetic involvement, angiotensin-II, a potent vasoconstrictor, can be expected to produce capsaicin-induced hypertension. However, our results exclude this possibility as losartan (AT_1-receptor antagonist) failed to block the hypertensive response as well as the apnea/bradypnea. In a study elsewhere, it has been shown that capsaicin can induce excitatory renal reflex in rats, resulting in sympathetic activation and pressor responses by involving different area of brain like solitary tract, rostral ventrolateral medulla and periventricular nucleus of hypothalamus [7, 8]. Direct modulation of respiratory neurons in the vicinity of these cardiovascular and sympathetic activity related brain areas could induce intermediate apnea/bradypnea response.

Recently, capsaicin is shown to release endothelin from sensory nerve terminals [15]. Endothelin is a peptide also produced by vascular endothelial cells and known to produce severe vasoconstriction thus may be responsible for capsaicin-induced hypertension and consequent respiratory response. In a study elsewhere, it has been shown that endothelin attenuated the capsaicin induced intermediate hypertensive response but the associated respiratory response persisted (1). Hence, the non-involvement of vasoconstrictor mediated hypertension and respiratory response is implicated.

In conclusion, capsaicin produces initial hypotensive, intermediate recovery and delayed hypotensive response. The initial hypotensive and apneic response is vagally mediated while the intermediate and delayed responses are not mediated through vagus. However, the intermediate hypertension was augmented after vagotomy and the associated respiratory response persisted. Intermediate hypertension in conjugation with respiratory response was not related to adrenergic or angiotensinergic mechanism. Thus, the respiratory response may be due to the direct action of capsaicin on central respiratory neurons.

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