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Superior laryngeal nerve section abolishes capsaicin evoked chemoreflex in anaesthetized rats

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Abstract. Respiratory effects of an intravenous injection of capsaicin were investigated in nine vagotomized and subsequently laryngeally deafferentated, urethane- and chloralose- anaesthetized and spontaneously breathing rats. Bolus injection of capsaicin (5 μg/kg) into the right femoral vein induced an expiratory apnoea of 4.23 ± 0.63 s duration (mean ± SEM). In post-apnoeic breathing, tidal volume increased by 14% from the control level ($P<0.05$) in all nine rats treated by vagotomy. Section of the superior laryngeal nerves (SLNs) precluded the occurrence of apnoea. Results of this study indicate that in vagotomized rats sensory input from the larynx constitutes an important pathway to the nodose ganglia endowed with capsaicin receptors.

Key words: control of breathing, rat, capsaicin, apnoea, laryngeal afferents

INTRODUCTION

Capsaicin, a selective neurotoxin for primary sensory neurones has been widely used to both: excitation and desensitisation of unmyelinated C and A_δ- fibres (Bevan and Szolcsányi 1990). Given to the right side of the circulation in cats and dogs capsaicin elicits the pulmonary chemoreflex consisting of prompt apnoea, bradycardia, hypotension, followed by rapid, shallow breathing. The effect was established to be connected with the stimulation of vagal afferent C-fibres in the pulmonary vascular bed and can be abolished by bilateral vagotomy (Coleridge et al. 1964, Makara and Győrgy 1967, Szereda-Przestaszewska and Wypych 1996). However, there is species variation in the response to capsaicin. Some studies in rats (Mitchell et al. 1984, Hedner et al. 1985, Paleček et al. 1989, Kaczyńska and Szereda--Przestaszewska 2000) and monkeys (Ravi and Singh 1996) showed that capsaicin administration following vagotomy could still produce pulmonary chemoreflex confined in cardiorespiratory sequeleae but with steadily occurring apnoea. This may suggest additional sites of capsaicin action, mainly on the extrapulmonary C-fibres. In our previous work (Kaczyńska and Szereda--Przestaszewska 2000) we have pointed out that the responses to capsaicin might be mediated by vanilloid (capsaicin-sensitive) receptors present in the nodose ganglia (Helliwell et al. 1998). In midcervically vagotomized rats the superior laryngeal nerve constitutes the main pathway to these ganglia. The present study started with the hypothesis that the arrest of breathing induced by capsaicin challenge into the pulmonary circulation is dependent on the integrity of the superior laryngeal nerves. This hypothesis has been tested by measuring ventilatory effects of capsaicin in initially vagotomized rats and after elimination of the superior laryngeal nerve input.

METHODS

Nine adult Wistar rats (weight 250-300 g) were anaesthetized with urethane (600 mg/kg) and alpha--chloralose (120 mg/kg) injected intraperitoneally, and placed in the supine position. The trachea was exposed in the neck, sectioned below the larynx and cannulated. Two catheters were inserted into the femoral vein and artery for drug administration and blood pressure recording, respectively. Rectal temperature was maintained at 38°C with a heating pad. The midcervical segments of the vagi and the superior laryngeal nerves (SLNs) at their entrance to the larynx were isolated and prepared for section later in the experiment. The experimental protocol has been approved by the local animal care committee.Tidal volume signals were recorded from the pneumotachograph (Electrospirometer C 56, Mercury) attached to the tracheal cannula. End-tidal CO_2 concentration was measured with a capnograph (Engstrom Eliza Plus, Gambro). Arterial blood pressure was measured with a pressure transducer (CK 01 Mera-Tronik) and blood pressure monitor (MCK 4011). Electromyogram of the costal diaphragm was recorded with bipolar electrodes, amplified (NL 104, Digitimer), filtered and measured with a model AS 101 (Asbit) leaky integrator (time constant = 100 ms). The recordings were registered on an Omnilight 8 M 36 aparatus (Honeywell). Capsaicin (8-methyl-N-vanillyl-6-nonenamide, Sigma) in a dose of 5 μg/kg dissolved in physiological saline supplemented with ethanol and Tween 80 (Coleridge et al. 1964) was injected as a bolus to the right femoral vein. The respiratory effects evoked by capsaicin challenge were recorded in (1) initially vagotomized animals and (2) following section of the superior laryngeal nerves. Each individual value of the tidal volume (V_T) was a mean taken during five consecutive breaths. Volume and the expiratory time component of the respiratory pattern were assessed just prior to the capsaicin injection, at the early post-apnoeic phase (the first five post-apnoeic breaths) and at 30 and 60 s after the challenge. The expiratory time (T_E) was determined from the record of integrated diaphragm activity. Prolongation of T_E was measured as the ratio of maximal T_E during post-capsaicin apnoea (T_{Ecaps}) to control expiration ($T_{Econtrol}$). All experimental data were analyzed by repeated measures two-way ANOVA with post-capsaicin time (0, early post-apnoeic phase, 30 s and 60 s) and innervation status (vagi cut and SLNs cut) as repeated measure factors, whereas T_E prolongation results were analyzed by repeated measures one-way ANOVA with innervation status as repeated measures factor. Significance of differences between individual experimental situations was evaluated by contrast analysis. In all cases, a $P<0.05$ was considered significant. The results shown are means ± 1 standard error.

RESULTS

Intravenous capsaicin injection at a dose of 5 μg/kg evoked sudden expiratory apnoea in all vagotomized

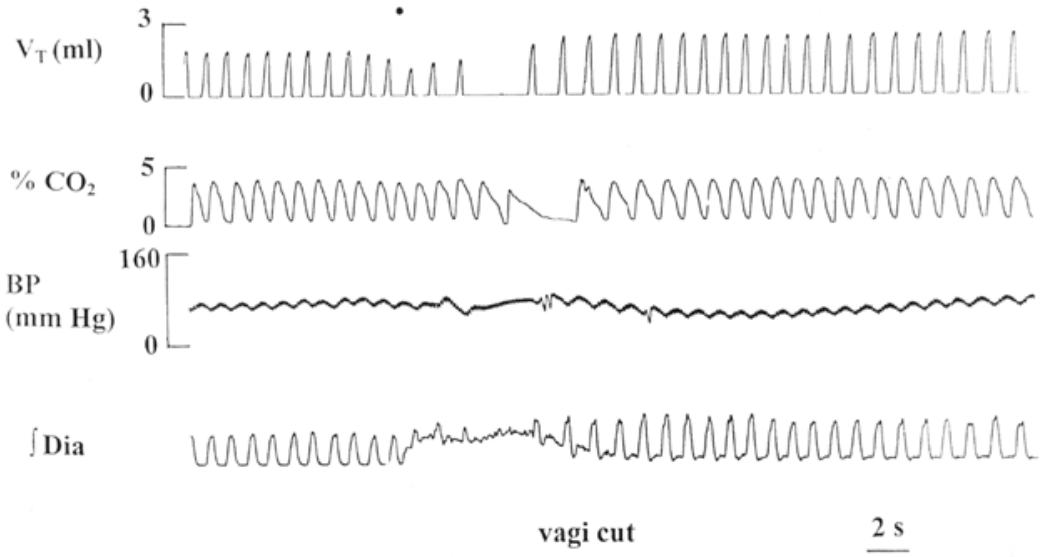

Fig. 1. Effects of intravenous capsaicin challenge on respiration-related indices in midcervically vagotomized rats. Capsaicin injection is marked by dot above the upper trace. Note: the expiratory apnoea coupled with tonic diaphragmatic activity followed by stimulated breathing. V_T, tidal volume; % CO_2, expiratory CO_2 concentration; BP, arterial blood pressure; Dia, integral of the diaphragmatic electromyogram.

rats. Figure 1 depicts a representative record of the respiratory response to capsaicin. During reinitiated breathing, tidal volume increased at 30 s and the effect persisted at 60 s after drug injection. Figure 2 shows the mean increase in tidal volume in response to capsaicin challenge in 9 vagotomized rats, subsequently treated by SLNs section. In the latter, the tidal volume did not change after drug injection. Respiratory effects of capsaicin were accompanied by an immediate and rapid fall in the blood pressure that persisted for not less than 30 s (Table I). As shown in Fig. 3, cutting of the SLNs resulted in the absence of the respiratory response to capsaicin administration. The mean prolongation of T_E (T_{Ecaps} /$T_{Econtrol}$) of 5.02 ± 0.49 in vagotomized rats was reduced to 1.05 ± 0.03 in SLNs' cut animals (P<0.0001) (Fig. 4). Rats with deafferentated larynges presented a short-lived decrease in the blood pressure confined to several seconds after the capsaicin challenge (Table I).

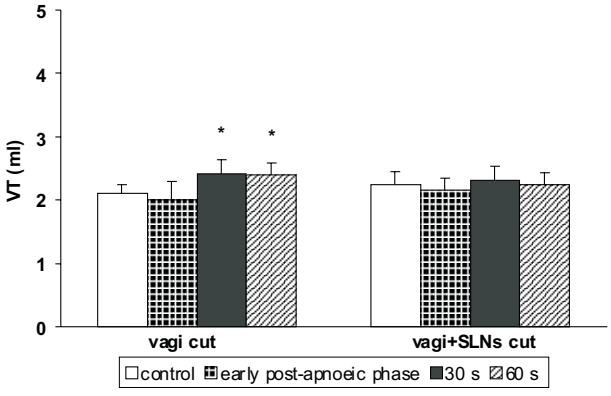

Fig. 2. Effect of i.v. capsaicin on tidal volume in vagotomized and subsequently SLNs-sectioned rats. All values are shown as mean ± 1 SEM, n = 9. Repeated measures two-way ANOVA has revealed a significant effect of capsaicin ($F_{3,24}$=5.5, P=0.004), no effect of SLNs section ($F_{1,8}$=0.00, P=0.93) and a trend for interaction between the effects of two repeated measure factors ($F_{3,24}$=2.74, P=0.06) on tidal volume. *, P<0.05 vs. the respective control (planned contrasts).

DISCUSSION

The results of this study confirm our hypothesis that superior laryngeal nerves contribute to the respiratory effects evoked by an intravenous capsaicin challenge. Compared to other species rats display a slightly different pattern of respiration in capsaicin-induced pulmonary chemoreflex, which is not always abolished by midcervical vagotomy (Hedner et al. 1985, Paleček et al. 1989, Kaczyńska and Szereda-Przestaszewska 2000). This phenomenon prompted us to search for other sites of capsaicin action, different from pulmonary C-fibres.

The present experiments confirmed the results obtained in our earlier study on capsaicin effects in rats (Kaczyńska and Szereda-Przestaszewska 2000). Ani-

Table I

Mean arterial blood pressure (MAP) changes induced by capsaicin challenge[a]

	Denervation status	Pre-capsaicin	Post-capsaicin		
			Early phase	30 s	60 s
MAP (mm Hg)	vagotomy	94.1 ± 6.2	80.5 ± 5.0**	76.7 ± 6.2**	88.5 ± 6.8
	vagotomy+SLNs cut	78.9 ± 6.7	68.3 ± 6.05**	77.4 ± 6.38	76.9 ± 5.7

[a], all values are means ± 1 SEM. of $n = 9$, two-way ANOVA has revealed significant effect of capsaicin ($F_{3,27}$=6.9, P=0.001), SLNs neurotomy ($F_{1,9}$=7.6, P=0.02) and significant interaction between the effects of the two main factors on MAP ($F_{3,27}$=3.7, P=0.02); **, P<0.01 vs. the respective pre-challenge value (planned contrasts).

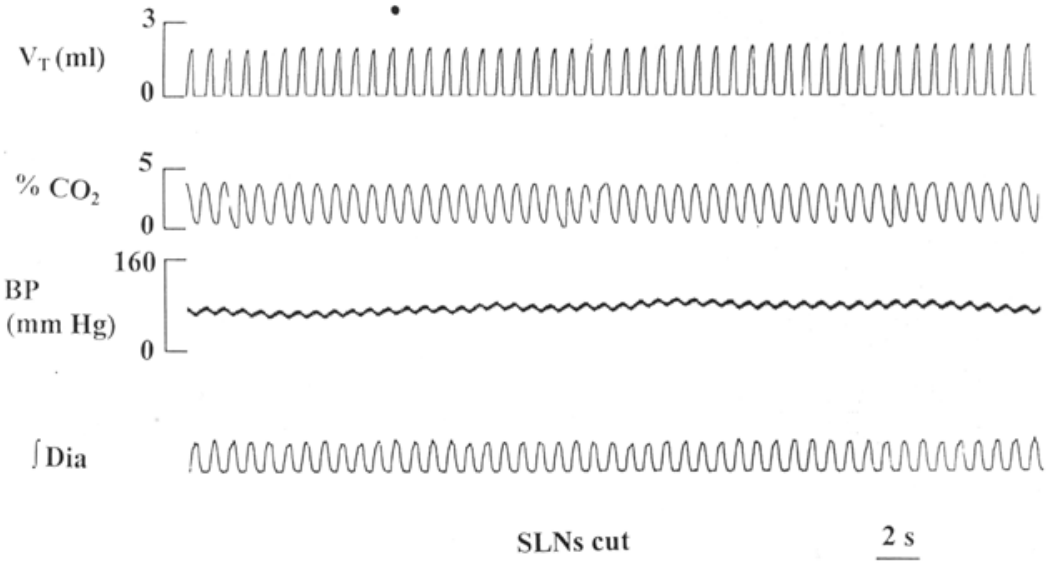

Fig. 3. Effects of intravenous capsaicin injection following SLNs section in the vagotomized rat. Capsaicin failed to induce any respiratory changes.

mals subjected to vagotomy answered with the same, already described pattern: the apnoea, subsequently increased tidal volume and a fall in blood pressure. The principal finding of the current study is that SLNs neurotomy eliminated respiratory inhibition. One possible explanation for this effect is that capsaicin injected into the right side of the circulation reaches the larynx and may excite vanilloid receptors present in the rat's airways and nodose ganglion (Szallasi et al. 1993, Helliwell et al. 1998) and most likely also on the superior laryngeal nerves. This is indirectly corroborated by

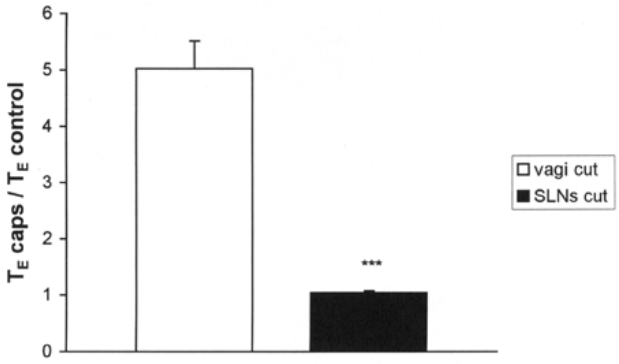

Fig. 4. Effect of SLNs section on capsaicin induced prolongation of expiratory time ($T_{E\ caps}$ / $T_{E\ control}$, mean ± 1SEM). Repeated measures one-way ANOVA has revealed a significant effect of SLNs neurotomy on T_E prolongation ($F_{1,9}$=87.1, P=0.0002). ***, P<0.001 vs. vagotomized rats (planned contrasts), $n = 9$.

the histological results showing that rat SLN consists of myelinated and numerous nonmyelinated capsaicin--sensitive fibres (Domeij et al. 1989). The potential importance of the superior laryngeal nerves in mediating capsaicin effects in rats might be associated with the presence of paraganglia possibly serving as peripheral chemoreceptors and containing neuropeptides (Dahlquist et al. 1994).

Activation of laryngeal sensory endings has an inhibitory effect on respiration resulting in protracted apnoea. The upper airway comprising larynx is a very potent reflexogenic area for triggering protective and defensive reflexes. This is due to the superficial location of C-fiber endings in the airway mucosa. Chemosensitivity of the larynx is well established, and capsaicin-sensitive nerve endings in guinea pigs and rats stimulated by instillation or inhalation of capsaicin evoke arrest of breathing. However, section of the superior laryngeal nerves does not preclude explicitly the apnoeic response. The response was described to be completely abolished (Tsubone et al. 1991, Lin et al. 2000) as well as reduced and not altogether eliminated (Paleček et al. 1990, Hishida et al. 1996). Despite these diverse effects of the disruption of the laryngeal input, the responses to local capsaicin challenge indicate their primary reflex origin from the larynx. The intravenous route of capsaicin challenge applied in our study would preferentially stimulate extrapulmonary, presumably laryngeal, C-fibres. This induces an immediate apnoea, most likely due to stimulation of vanilloid receptors in the nodose ganglia. Our report is the first to compare the respiratory response to intravenous capsaicin in vagotomized and subsequently SLNs-sectioned rats. From our finding that the section of the superior laryngeal nerves unfailingly precluded the apnoeic effects of capsaicin (Figs. 3 and 4), it may reasonably be concluded that the apnoea was due to activation of laryngeal afferent pathways. In general agreement with the previous works (Kamosińska and Szereda-Przestaszewska 1988, Mortola and Rezzonico 1989), deafferentation of the larynx did not significantly modify the tidal volume (Fig. 2). Having current results in hands we hesitate to unequivocally ascribe post-capsaicin increase in tidal volume to the intact SLN input. However, as shown in Fig. 2, the interaction between denervation status and capsaicin challenge approaches statistical significance (P=0.06), which suggests that SLNs section affects the response to capsaicin.

The superior laryngeal nerve in the rat was shown to convey stretch receptor and aortic baroreceptor impulses (Andrew 1956). Although SLNs section eliminated apnoeic spells, the fall in mean arterial pressure was short-lived compared to the animals treated solely by vagotomy (Table I). This finding supports our earlier prediction (Kaczyńska and Szereda-Przestaszewska 2000) that capsaicin can affect blood pressure by stimulating brainstem reflex centre or carotid sinus baroreceptors (Holzer 1988). It should be also remembered that SLNs' neurotomy at the C_2-C_3 level performed in the current experiments does not exclude the baroreceptor aortic pathway in the rat, which may well contribute to the hypotensive response.

CONCLUSION

In summary, our results show that in the rat, apnoea evoked by an intravenous capsaicin administration is mediated by capsaicin-sensitive fibres in the superior laryngeal nerves reaching the nodose ganglia.

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