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Respiratory effects of capsaicin occur beyond the lung vagi in anaesthetized rats

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Abstract. The effects of an intravenous capsaicin challenge on the respiratory pattern and ventilation were studied in 15 urethane/chloralose-anaesthetized, spontanously breathing rats. Bolus injection of capsaicin at a dose of 5 μg/kg into the right femoral vein evoked respiratory arrest in all animals (both prior to and after bilateral midcervical vagotomy), which effect was abolished by ruthenium red pretreatment. Breathing that followed the apnoea was of enlarged tidal volume and initially increased respiratory rate, which resulted in an augmented ventilation. The capsaicin-induced respiratory changes were independent of vagal integrity and may depend on stimulation of vanilloid receptors within the nodose ganglia.

Key words: control of breathing, rat, capsaicin, apnoea, vagus nerve, vanilloid receptors, ruthenium red

INTRODUCTION

Intravenous administration of capsaicin to cats and dogs elicits the pulmonary chemoreflex consisting of prompt apnoea, bradycardia and hypotension, followed by rapid shallow breathing (Pórszász et al. 1955, Toh et al. 1955, Coleridge et al. 1964). The response has been suggested to be mediated by vagal afferent C-fibres in the pulmonary vascular bed (Coleridge et al. 1964), vagotomy or cooling of the vagi abolishes the effect of intravenous capsaicin injection (Pórszász et al. 1955, 1957, Toh et al. 1955, Coleridge and Coleridge 1984, Kopczyńska and Szereda-Przestaszewska 1998).

Rats have been claimed to present the same pattern of pulmonary chemoreflex (Makara et al. 1967). However, in contrast to the extensive studies performed in cats and dogs, the relevant data on rats are scarce. The ventilatory response to capsaicin was only evaluated in two studies during the post-apnoeic period. One described a tendency for tidal volume reduction and shortening of the inspiratory duration that was precluded by vagotomy (Mitchell et al. 1984). Hedner et al. (1985) reported no ventilatory effect 3 min after capsaicin challenge in the intact rat. Inspection of the records presented by Lee and Lundberg (1994) shows an initial decrease in tidal volume just after the apnoea, followed by normalization during capsaicin-triggered rapid breathing, and no response in vagotomized rats.

Most studies agree that vagotomy precludes cardiovascular effects of capsaicin in rats (Makara et al. 1967, Hedner et al. 1985, Chahl and Lynch 1987, Lee and Lundberg 1994). On the other hand, high capsaicin dose (30 μg/kg) was shown to decrease blood pressure after vagotomy (Hedner et al. 1985). Moreover, in some vagotomized rats 5 μg/kg (Paleček et al. 1989) or higher (Mitchell et al. 1984, Hedner et al. 1985) intravenous capsaicin doses provoked an expiratory apnoea. The discrepancy between earlier studies concerning the respiratory effects of capsaicin prompted us to further investigate the acute effects of injections in the intact and vagotomized rats. Some of our preliminary results have been reported earlier (Kaczyńska 1999).

METHODS

The experimental animal procedures used in this study has been approved by the local animal care committee. A total of fifteen male Wistar rats, weighing 260-300 g, were anaesthetized with an intraperitoneal injection of urethane (600 mg/kg) and chloralose (120 mg/kg). Animals were placed supine, a cervical tracheostomy was performed and the trachea cannulated low in the neck. Body temperature was maintained at 38°C with a heating pad. The femoral vein and artery were catheterized for drug administration and blood pressure recording, respectively. The midcervical segments of vagal nerves were isolated and prepared for cutting. A pneumotachograph (Electrospirometer C 56, Mercury) was attached to the tracheal cannula and the flow signal or tidal volume was recorded. End-tidal CO_2 was measured continuously 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).

Capsaicin (8-methyl-N-vanillyl-6-nonenamide, Sigma) was dissolved at a concentration of 5 μg/ml in ethanol- and Tween 80-supplemented physiological saline (Coleridge et al. 1964). The drug was administered as a bolus dose of 5 μg/kg (Paleček et al. 1989) to the right femoral vein. The respiratory effects of capsaicin challenge were recorded in (1) neurally intact rats, (2) after midcervical vagotomy, and (3) after intravenous administration of 1 mg/kg ruthenium red (non-competitive capsaicin antagonist) (Naida et al. 1996) to midcervically vagotomized rats. Each individual value of the ventilatory parameters studied (V_T, V_E, and f) was a mean taken during five consecutive breaths. The ventilatory parameters 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 tidal airflow. Prolongation of T_E was measured as the ratio of maximal T_E during post-capsaicin apnoea (T_{Ecaps}) to control expiration ($T_{Econtrol}$). The duration of apnoeic period in expiratory airflow was measured as the time of apnoea. All experimental data were analysed by repeated measures two-way ANOVA with post-capsaicin time (0, early post-apnoeic phase, 30 s and 60 s) and innervation status as (intact, vagi cut, and vagi cut + RR pretreatment) repeated measure factors, whereas T_E prolongation results were analysed by repeated measures one-way ANOVA with innervation status as repeated measures factor. Significance of differences between individual experimental situations was evaluated by planned contrasts. In all cases, a $P<0.05$ was considered significant.

RESULTS

Intravenous capsaicin challenge produced uniform cardiorespiratory effects, comprising an apnoea followed by stimulated breathing and a fall in systemic blood pressure, in all rats. There was no respiratory effect when similar volumes of the solvent were administered. Capsaicin injected at a dose of 5 μg/kg evoked in all rats while intact and subjected to vagotomy the expiratory apnoea of mean duration of 4.5 ± 1.85 s and

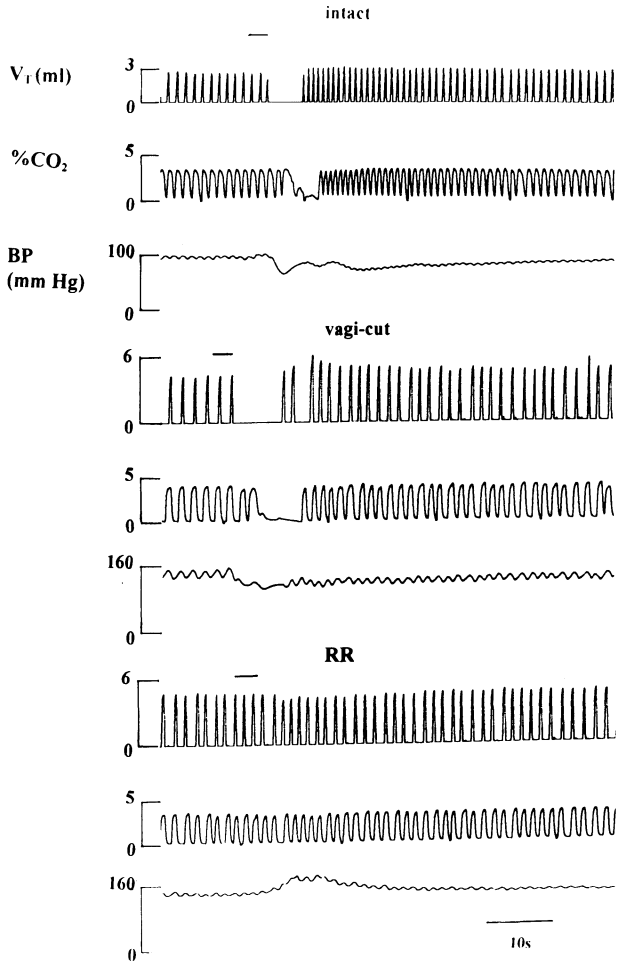

Fig. 1. Effects of intravenous capsaicin challenge on selected respiration-related indices in intact, midcervically vagotomized, and ruthenium red- (RR) treated midcervically vagotomized rats. Capsaicin injection is marked by a dash above each record. Note the expiratory apnoea followed by stimulated breathing in the intact and vagotomized rat and the absence of any respiratory response after RR treatment. V_T, tidal volume; % CO_2, expiratory CO_2 concentration; BP, arterial blood pressure.

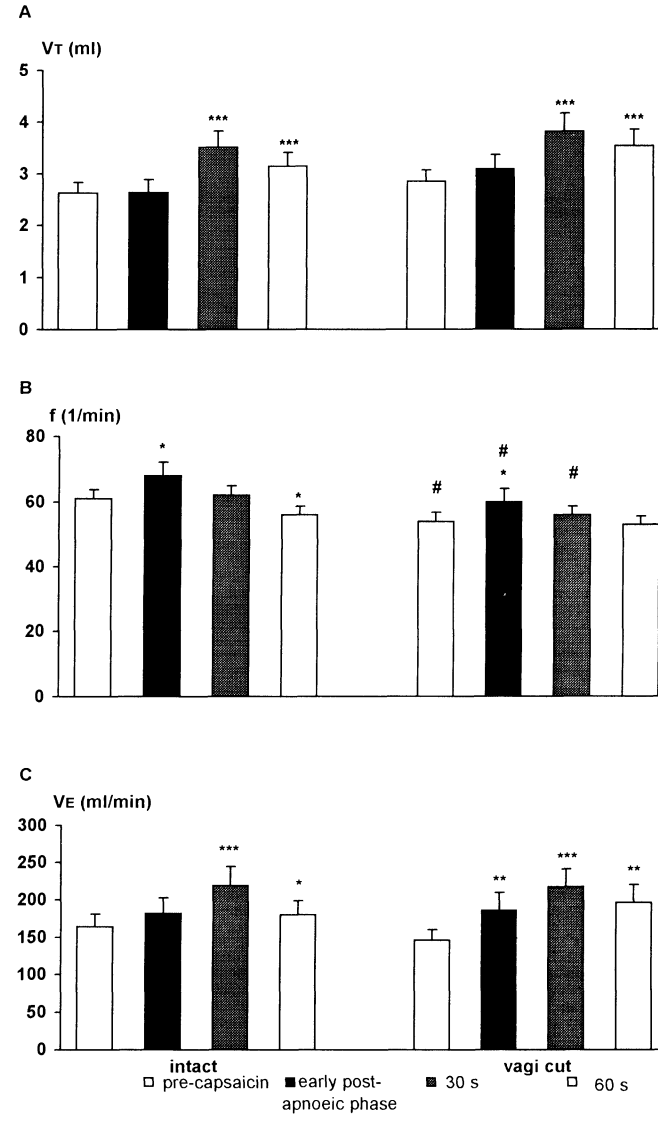

Fig. 2. Effect of i.v. capsaicin and vagotomy on tidal volume (V_T, panel A), respiratory rate (f, panel B), and minute ventilation (V_E, panel C) in the rat. All values of the respiratory parameters are shown as mean ± S E. A, repeated measures two-way ANOVA has revealed a significant effect of capsaicin ($F_{3,42}$ =15.8, P<0.000001), no effect of vagotomy ($F_{1,14}$ = 3.4, P=0.08) and insignificant interaction between the two repeated measure factors ($F_{3,42}$ = 0.8, P=0.4) on tidal volume. B, the two-way ANOVA has revealed significant effects of capsaicin ($F_{3,42}$ = 10.9, P=0.00002) and denervation ($F_{1,14}$ = 5.4, P=0.03), but no significant interaction between the two repeated measure factors ($F_{3,42}$ =1.6, P=0.2) on respiratory rate. C, the two-way ANOVA has revealed a significant effect of capsaicin ($F_{3,42}$ =14.0, P<0.000002), no effect of vagotomy ($F_{1,14}$ = 0.0, P=1.0) and insignificant interaction between the two repeated measure factors ($F_{3,42}$ = 1.3, P=0.3) on minute ventilation. *P<0.05, ** P<0.01, *** P<0.001 vs. the respective control, # P<0.05 vs. the corresponding value in neurally intact rats.

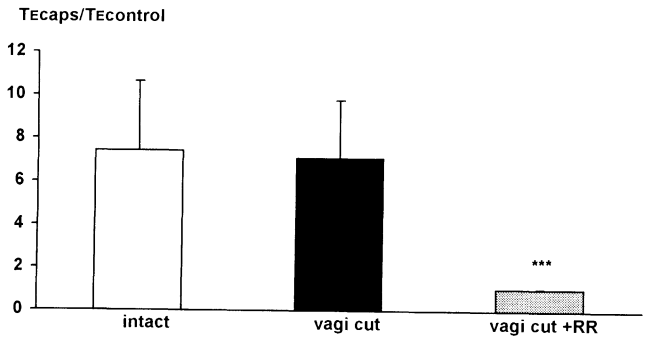

Fig. 3. Effects of vagal section and ruthenium red (RR) administration on the capsaicin-induced prolongation of expiratory time ($T_{E\ caps}/T_{E\ control}$, mean ± S E). Repeated measures two-way ANOVA has revealed a significant effect of ruthenium red on T_E prolongation ($F_{2,16}$=19.2, P=0.00005). *** P<0.001 vs. either of the other two experimental conditions (planned contrasts), n = 9.

4.9 ± 1.91 s (n = 15). Figure 1 depicts a typical response to intravenous administration of capsaicin in the rat while neurally intact, vagotomized and treated with ruthenium red (RR). In both neural states, the arrest of breathing was acompanied by the fall in blood pressure. After resumption of breathing, tidal volume and respiratory rate increased. Injection of capsaicin 2 min after intravenous RR administration did not affect the cardiorespiratory variables.

Mean changes in tidal volume during control and post-capsaicin breathing are shown in Fig. 2A. The increase in tidal volume in vagotomized rats was not statistically significant (P=0.08). Tidal volume did not change at the initial phase of resumed breathing compared to the respective control value, but increased at 30 and 60 s both in intact and vagotomized rats. Compared to the respective control, the respiratory rate increased after resumption of breathing in both neural states and remained elevated at 60 s in the intact rat (Fig. 2B). Minute ventilation was increased at 30 and 60 s following capsaicin injection in both neural states (Fig. 2 C). As shown in Fig. 3, prolongation of T_E was virtually identical in intact and vagotomized rats, and was abolished by vanilloid receptors' blockade. Mean arterial pressure fell during the arrest of breathing in both vagotomized and intact rats and normalized at 60 s in these latter, whereas it normalized already at 30 s post-capsaicin in vagally deafferentated rats (Table I).

DISCUSSION

The pattern of breathing that followed capsaicin challenge was somewhat different from the classical response reported in cats, dogs and rats. This study showed augmentation of tidal volume at 30 and 60 s after capsaicin administration (Fig. 2A), whereas an earlier study has shown a decrease in tidal volume (Mitchell et al. 1984). The reason for this discrepancy is not clear but may be related to the much higher dose of capsaicin used in that previous study. Timing component of the breathing pattern in the current experiments displayed modest but highly consistent increments immediately after

TABLE I

Effect of intravenous capsaicin administration on mean arterial pressure (MAP) in the intact and vagotomized rats

	MAP (mm Hg)[a]			
	Post-capsaicin time			
	0	apnoea	30 s	60 s
Intact	99.6 ± 6.9	86.8 ± 7.1***	90.0 ± 6.8**	97.7 ± 7.8
Vagotomized	110.6 ± 6.9	102.0 ± 6.7**	101.0 ± 7.2	103.1 ± 7.4

[a] Values are means ± SEM, n = 15; two-way ANOVA has revealed significant effect of capsaicin ($F_{3,42}$ = 6.6, P= 0.0009), no effect of denervation ($F_{1,14}$ = 4,3, P=0.05) and no interaction between the two repeated measure factors ($F_{3,42}$ = 1,4, P=0.2); ***P< 0.001, **P<0.01 compared to control values, (planned contrasts).

reinitiation of breathing (Fig. 2B). The enhanced minute ventilation, for the most part, matched the increases in tidal volume rather than decreased breath cycle duration.

Anaesthesia and rat strain used in the present study were the same as those used by others (for references see Introduction). Nonetheless, we have also tested a number of animals under thiopental anaesthesia and found they responded to capsaicin with increased tidal volume as well. We did not observe differences in the sensitivity of individual animals to capsaicin. Moreover, some of them treated with an intravenous serotonin showed a typical chemoreflex response of decreased tidal volume and tachypnoeic breathing. Evidently, the enlarged tidal volume was a characteristic feature of the respiratory response to capsaicin in our rats. The respiratory response to capsaicin took also place following section of midcervical vagi.

The dose of capsaicin used in the present study (5 μg/kg) was selected because the same (Paleček et al. 1989, Naida et al. 1996) or smaller doses (Makara et al. 1967, Hedner et al. 1985, Lee and Lundberg 1994) have been previously used to evoke pulmonary chemoreflex in the rat. It is well established that low capsaicin doses (few μg per kg) stimulate only the smallest chemosensitive C-fibres in the rat, mouse and guinea pig (Holzer 1988). Enhanced traffic from vagal C-fibres was shown to initiate a reflex that increases breathing frequency and decreases tidal volume (Schelegle et al. 1995).

At the dose used in the present experiments capsaicin induced immediate expiratory apnoea followed by stimulated breathing of increased tidal volume and respiratory rate. The respiratory response triggered in our study did not quite match the typical constellation of the pulmonary chemoreflex. Both capsaicin-affected components of the respiratory pattern are regulated by two mechanisms: the volume feedback from the lungs and bulbopontine mechanisms (Sant'Ambrogio and Sant'Ambrogio 1997). In the intrapulmonary chemoreflex, C-fibres contribute to both the afferent and efferent limits of the reflex (Coleridge and Coleridge 1994). In the present study, however, we have demonstrated that capsaicin still evoked respiratory arrest followed by stimulated breathing after interrupting afferent vagal input to the medullary centres. Therefore, the stimulated breathing must be regulated by the activity of bulbopontine structures.

There are several possibilities worthy of consideration in finding the afferent inputs for the response to capsaicin in vagotomized rats. Capsaicin was described to exert its respiratory effects partly through the superior laryngeal nerves in the guinea pig and rat (Tsubone et al. 1991, Naida et al. 1996), this afferent fibre path to the nodose ganglia was preserved in our experiments. Capsaicin sensitive afferents belonging to trigeminal, vagal and glossopharyngeal nerves were found in the spinal trigeminal nuclei, solitary tract and nucleus commissuralis (Jancsó and Király 1980). They innervate the mucous membranes of the nose, laryngeal and pharyngeal tissues. Trigeminal afferents in the nose were described to react to capsaicin in the guinea pig (Sekizawa and Tsubone 1994). Conceivably, intravenous capsaicin could contribute to central transmission and excitation of the respiratory centres through its afferents in the reflex-ogenic areas of the Vth, IXth and Xth nerves in the rat.

To the best of our knowledge, there is no report on the effects of capsaicin on peripheral chemoreceptors. Hedner et al. (1985) using 30 μg/kg of capsaicin showed that the reduced apnoeic response to capsaicin in vagotomized rats was not affected by consecutive sectioning of the glossopharyngeal nerves. We are hesitant to ascribe the capsaicin-induced breathing arrest to the central origin. Intracerebroventricular injections of capsaicin do not evoke apnoea in the rat (Hedner et al. 1985). One likely explanation is that the site where the respiratory arrest intervenes are nodose ganglia. As previously mentioned, the afferent path to the ganglia *via* the superior laryngeal nerves was left intact in our experimental design. However, a more likely possibility is that capsaicin carried in the blood-stream actively stimulates the nodose ganglia that abund with vanilloid receptors in the rat (Szallási et al. 1995, Wood and Docherty 1997, Helliwell et al. 1998). The present study argues for the latter as the blockade of vanilloid receptors with RR precluded the effects of capsaicin. It has been recently reported that this inorganic dye, a calcium channel antagonist, can inhibit the capsaicin-induced cardiorespiratory effects both *in vitro* (Lou et al. 1991) and *in vivo* (Petho and Szolcsányi 1990, Naida et al. 1996).

Depressor response during post-capsaicin apnoea and post-apnoeic breathing we observed is well established and described as vagally dependent in the rat (Makara et al. 1967, Hedner et al. 1985, Chahl and Lynch 1987, Lee and Lundberg 1994). Some studies point out, however, that higher dose of capsaicin applied after vagotomy could decrease blood pressure even further (Makara et al. 1967, Hedner et al. 1985). Capsaicin is known to easily cross the blood-brain barrier (Saria et al. 1982, Reid and McCullogh 1987). Therefore, the most plausible ex-

planation for the post-capsaicin fall in blood pressure in vagotomized rats is a direct effect of capsaicin on the brainstem reflex centre that decreases blood pressure by releasing substance P (Donnerer and Lambeck 1983), and/or to capsaicin action on cerebral vessels innervated by capsaicin-sensitive fibres (Holzer, 1988). Alternatively, depressor effects of capsaicin may be attributed to its action on the carotid sinus baroreceptors (Pórszász et al. 1957, Brender and Webb-Peploe 1969). Afferentation from this region was preserved intact in our experiments.

In conclusion: Evidence has been provided that the apnoea triggered by intravenous capsaicin injection and the stimulated breathing of increased tidal volume that follows are carried out beyond the lung vagi in the rat. These respiratory responses seem to be mediated by the vanilloid receptors in the nodose ganglia as they are abolished by RR pretreatment.

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