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EXPERIMENTAL STUDY

Cough threshold and reactivity to mechanical stimulation of the trachea in the rabbit Preliminary observations

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Abstract: *Objectives:* The aim of the study was to characterise mechanically induced cough threshold and reactivity by exposing the trachea to stimuli of variable duration in rabbit.
Background: Long lasting mechanical stimulation is widely used in experimental protocols studying cough reflex. The cough threshold and reactivity to chemical agents is known to change due to e.g. airway inflammation but similar evidence for mechanical stimulation has not been reported.
Methods: The tracheal provocation was realized in two anesthetized tracheotomized rabbits with a rotating probe actuated by a small electrical motor with mechanical stimulus times (ST) lasting 50, 150, 300 and 600 ms. Cough reflex was evaluated from tidal volume and airflow signals.
Results: The incidence of cough reflex (single or multiple) increased from 8 % (ST 50ms) to 84 % (ST 600 ms). With the lengthening of stimulus, the rate of multiple responses increases.
Conclusion: The technique developed here may prove useful to standardize the protocols of mechanical cough in the experimental animal using an approach similar to chemical tussigenic agents. The cough threshold could be defined as minimal ST (STmin) capable to elicit 1 cough and cough reactivity obtained by interpolation as ST that provokes 50 % of cough responses (ST_{50}) *(Tab. 1, Fig. 3, Ref. 27).* Full Text in free PDF *www.bmj.sk.*
Key words: cough reflex, mechanical stimulation.

The mechanical stimulation of sensory terminals has been an important component of experimental protocols studying defensive responses elicited from various levels of the airways for more than 100 years (1–3). A large number of studies have been provided to enlarge the knowledge in the field of airway defence mechanisms, especially the cough reflex (CR). A review of the literature showed that the duration of mechanical stimuli used in most studies exceeded several seconds (usually 5–20 s) and resulted mostly in multiple defensive responses consisting of coordinated occurrence of CR and expiration reflex (ER) (4–8). The quantitative analysis of pleural pressure, airflow and/or abdominal muscle EMG recordings now allows to differentiate CR from ER and to characterize their frequency and intensity in various experimental studies separately in the means of their physiology, physiopathology and pharmacology (4–5, 8–13).

Long-lasting mechanical stimulation produces defensive responses attesting that activation of sensory nerve terminals has been strong enough to initiate afferent action potential discharge travelling to the brainstem where a motor pattern is activated. In order to assess the effect of various conditions such as airway inflammation, a dose – response relationship has been described to assess chemically-induced CR. Therefore, the use of a cough threshold has been described that characterize the reactivity to inhaled tussigenic agents, such as the concentration that causes two (C_2) or five (C_5) coughs (14–16). We failed to find any reported evidence determining similar threshold and reactivity studies for cough induced by mechanical stimulation. We reasoned that the latter could be established by exposing the airways to mechanical stimuli of variable duration.

The aim of the study was to provide a model allowing to characterize mechanically induced tracheal CR in the rabbit and to assess cough threshold and reactivity of the trachea to mechanical stimulation.

Material and methods

General

Two New Zealand adult rabbits were anaesthetized with a mixture of urethane (500 mg/kg), alpha-chloralose (50 mg/kg) and sodium borate (50 mg/kg) injected through an ear vein. Supplemental doses were given every 2 hours. Rectal temperature was continuously monitored and maintained at 38 °C. The rabbit was tracheostomized and intubated with a tracheal steel cannula. Animal care and experiments were performed according to recommendations 86-609 CEE issued by the Council of

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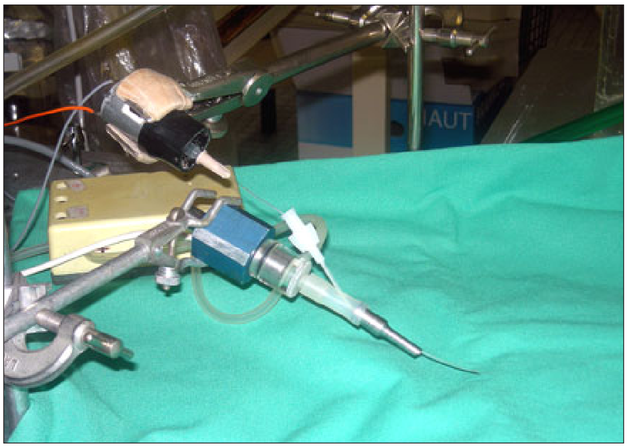

Fig. 1. The system developed for mechanical stimulation of trachea. The rotating probe consisted of the silastic semi-rigid catheter (1) with proximal end connected to a small electrical motor (2) and the distal end placed into the rabbit trachea through the tracheal cannula (3). Fleisch pneumotachograph (4).

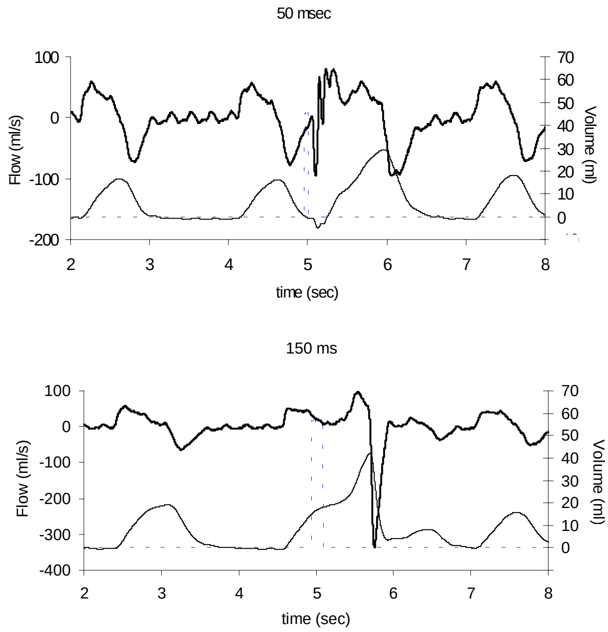

Fig. 2. Airflow (upper trace) and tidal volume signals (lower trace) showing defensive responses provoked by stimulus duration of 50 ms and 150 ms. Top: expiration reflex elicited with 50 ms stimulus duration during expiration, characterized by an increased expiratory flow not preceded by an increase in VT. Bottom: single cough reflex elicited with probing duration of 150 ms (increase in both VT and V'Emax).

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Tracheal stimulation

An apparatus was developed to stimulate the lower end of the trachea for short periods of time (Fig. 1). It was thought that a rotating probe actuated by a small electrical motor could ensure mechanical stimulus time (ST) as short as 50 msec, with sharp on- and off turning. The electrical engine (low voltage DC motors 719RE280, MFA/Comodrills, UK) has an outer diameter of 23.8 mm, operates under 3 V and develops a speed of 9200 rpm under no load, 7800 rpm and a torque of 20 g.cm at maximal efficiency. A silastic semi-rigid catheter (1.2 mm OD) was snugly fit onto the 2 mm engine shaft and the distal end introduced 3 cm downward into the tracheal cannula. There was a tight fitting between the catheter and the cannula (Fig. 1) and preliminary measurements of the animal mechanical respiratory impedance showed similar values with and without insertion of the catheter. When actuated, the engine would spin the catheter and rub its tip onto the airway mucosa. The engine insured a rectangular pattern to the mechanical stimulus, particularly the immediate cessation of catheter rotation at off-turning. Hence a discrete mechanical stimulus with well defined temporal course could be obtained. The electrical signal from the rotating engine fed to the computer together with the respiratory signals and allowed an accurate identification of stimulus time course in offline analysis.

Protocol

Mechanical stimulations of the trachea were performed during quiet breathing, uninterrupted for at least 1 min. The following stimulus ST's were used: 50 ms, 150 ms, 300 ms and 600 ms. Different ST's were applied in inspiration and expiration, in a pseudo-random order. Approximately 24 trials were aimed in each rabbit.

Visual inspection of the trachea with the stimulation catheter in place after the experiment on autopsy showed the probing tip ca 1 cm above the carina. There was no macroscopic evidence of epithelial damage.

Ventilatory responses

Flow was measured at the tracheal opening using a Fleisch # 0 pneumotachograph (Metabo, Hepalinges, Switzerland). The flow signal was digitized at 160 Hz, fed to a computer and integrated to volume. Tidal volume (V_T) and flow were displayed breath by breath and stored on disk for later analysis. Cough responses to tracheal stimulation were identified during off-line analysis by differentiating them from ER as follows: the respiratory cycle undergoing the stimulation (stimulation breath) was compared with the 2 preceding respiratory cycles (reference breaths) for a change in V_T and maximal measured expiratory flow (V'Emax). CR was defined by an appreciable increase in both V_T and V'Emax and was differentiated from (ER) defined as increase in V'Emax not preceded by a significant increase in V_T, which means that V_T value of stimulation breath is higher then mean V_T +3SD of 2 reference breaths. To asses cough thresh-

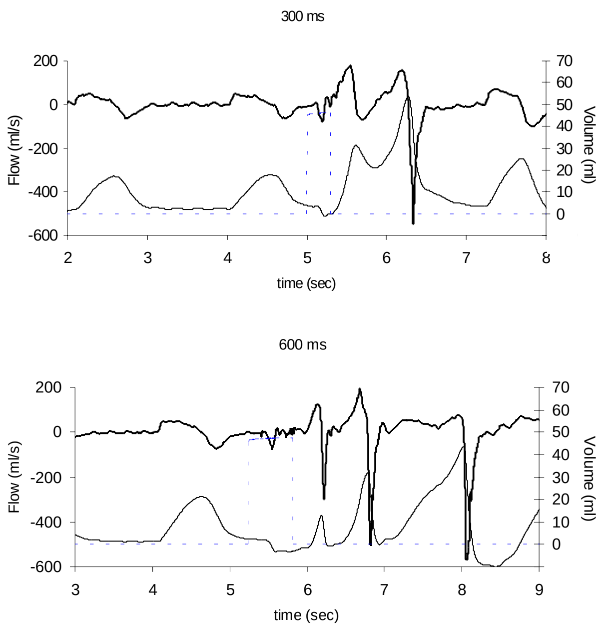

Fig. 3. Airflow (upper trace) and tidal volume signals (lower trace) showing defensive responses provoked by stimulus duration of 300 ms and 600 ms. – Top: 300 ms stimulation time provoked the expiration reflex (increased V'E max not preceded by an increase in VT) followed by the cough reflex (increase in both VT and V'Emax). Bottom: 600 ms stimulation time elicited two expiration reflexes followed by two cough reflexes.

old and reactivity of the trachea to mechanical stimulation only cough reflex containing responses were further analysed. They occurred as single CR (a single cough response to mechanical stimulation) (Fig. 2) or multiple response (several expulsive efforts of CR´s or CR's preceded by ER's) (Fig. 3).

Data analysis

Two time two cross tabulation was used to test the association between type of responses (single vs. multiple responses) and ST, and phases of the breathing cycle (inspiration, expiration). As the expected cell sizes were small, Fisher exact test was used.

Results

The short mechanical stimulation provoked CR as single or multiple events (Figs 2, 3).

No cough response was seen in 11/12 attempts (92 %) performed with ST 50 ms, 7/12 (58 %) with ST 150 ms and 4/12 (33 %) with ST 300 ms and 2/12 (17 %) with ST 600 ms (Tab. 1). A significant relationship between the ST and incidence of "no cough response attempts" was observed (p=0.05).

The incidence of single CR had an increasing trend from 1/12 (8 %) with ST 50 ms, 3/12 (25 %) with ST 150 ms, to 5/12 (42 %) with both ST 300 ms and ST 600 ms (Tab. 1).

Tab. 1. The incidence of cough reflex (CR) responses provoked by different stimulation time (ST).

ST	50 ms	150 ms	300 ms	600 ms
No CR response	11	7	4	2
Single CR	1	3	5	5
Multiple response	0	5	2	3
Total	12	12	12	12

Multiple defensive responses were not elicited with ST 50 msec, but their frequency was gradually increasing with ST: 2/12 (16 %) at 150 ms; 3/12 (25 %) at 300 ms and 5/12 (42 %) at 600 ms (Tab. 1).

A significant relationship between the phase of the stimulation timing within the breathing cycle and single vs. multiple responses was observed (p=0.011). A single CR was elicited significantly more often in inspiration compared to expiration (p=0.03) whereas multiple responses (beginning with ER) tended to be elicited significantly more often during expiration (p=0.058) compared to inspiration.

Discussion

The study shows that a mechanical stimulus – ventilatory response relationship may be described using a range of tracheal stimulation durations. It is also suggested that the excitation of receptive fields of the tracheal cough receptors elicit different response when the stimulation is limited to inspiration or expiration.

The mechanical stimulus was standardized for duration, onset and offset, with the particular aim to define a threshold response. Transducing a number of probe rotations into a cough receptor volley may depend on mechanical factors that eventually determine the stimulus intensity applied to the receptive field. The tracheal diameter may vary slightly from one rabbit to another, as well as along the breathing cycle, so that probing the airway surface may be associated with different torque, hence different mechanical stress. On the other hand, the characteristic cough receptor has a rapid adaptation, which makes it most sensitive to even lightest but varying mechanical stimulation (17). Present results are in agreement with recently published paper demonstrating that CR is favoured in inspiration that could result from the modulation of mechanosensitivity of afferent neurons and/or integrative processes in brain stem during breathing cycle (18).

The airway responses description was limited to airflow and volume patterns, together with the auditory identification of forced expired flow associated with CR. The model could of course be implemented with respiratory parameters such as pleural pressure or respiratory muscle EMG activity, in an attempt to better differentiate CR from ER, which have different integrative and efferent pathways (4, 19–23). The procedure of airflow monitoring in the airway on the other hand has the advantage to be simple and fast, therefore not requiring additional or prolonged anaesthesia. Because the latter is determinant to CR (24, 25), its level should be as constant as possible throughout a given experiment and comparable among animals. Furthermore, the qualitative estimation of tidal volume and expiratory flow was found reliable in distinguishing CR from expiration reflex (26, 27) and the expiratory sound was useful in differentiating a cough effort from an occassional deep breath.

The technique developed here may prove useful to standardize protocols of mechanical CR assessment in the experimental animal using an approach similar to chemical tussigenic agents (15). The cough threshold and reactivity of the trachea to mechanical stimulation for instance could be assessed from data plots such as those presented in Figs 2 and 3.

The cough threshold would be the minimal stimulus time (ST_{min}) that is capable to elicit 1. In the current study, ST_{min} would be 50 msec in one animal and 150 msec in the other. Reactivity could be obtained by interpolation, as the stimulus duration that provokes 50 % of cough responses (ST_{50}). Each rabbit here was stimulated on 6 occasions at each ST. In the first rabbit, the cough incidence was 3/6 at ST 150 ms. The corresponding ST_{50} was therefore 150 ms. In the second rabbit, there were 0/6 and 4/6 coughs at 50 ms and 150 ms, respectively, resulting in ST_{50} = 100 ms.

Thus the quantitative description of CR may be extended to another type of irritation in the experimental animal. The method provides an opportunity to stimulate during either phase of the breathing cycle and has therefore the potential to study the physiological mechanisms of CR dependent on breathing. Such information may of course not be gained when tussigenic agents are inhaled because the airways are exposed during both inspiration and expiration. It is interesting that single CR was elicited mostly with the stimulus given in inspiration and that expiration favoured multiple responses containing both expiration reflex and CRs. These observations require further experimental work. Finally, the set-up could easily be applied to characterize ventilatory responses to mechanical irritation of the airways in patho-physiological integrated animal models such as induced airway inflammation or pharmacological studies of down regulation of cough.

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