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Differential effect of codeine on coughs caused by mechanical stimulation of two different sites in the airway of guinea pigs

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Abstract

We studied the difference in the effects of codeine on coughs caused by mechanical stimulation to the larynx and to the bifurcation of the trachea in lightly anaesthetized guinea pigs. Mechanical stimulation to the larynx or the bifurcation of trachea caused a stable cough response. The response was reproducible over 60 min, when stimulation was repeatedly applied at 20-min intervals. No significant difference was found between the amplitudes of the responses to mechanical stimulation of the larynx and of the tracheal bifurcation. Codeine, 10, 20 and 50 mg/kg, dose dependently depressed the coughs caused by larynx stimulation. The antitussive, however, failed to depress the cough caused by stimulation to the tracheal bifurcation, although a large dose, 50 mg/kg, significantly depressed the cough. In capsaicin-treated guinea pigs, codeine at 20 mg/kg significantly depressed the cough caused by stimulation to the tracheal bifurcation. The present results suggest that cough caused by mechanical stimulation to the larynx might be more sensitive to codeine treatment than cough caused by stimulation to the bifurcation of trachea. Furthermore, it is suggested that coughs caused by mechanical stimulation to both sites might consist of at least two components as regards their pharmacological nature. © 1997 Elsevier Science B.V.

Keywords: Codeine; Larynx; Trachea bifurcation; Mechanical stimulation; Cough, codeine-resistant; C-fiber; A-fiber

1. Introduction

Coughing basically functions to keep the airway normal and clean by clearing excessive mucus production and foreign substances that have been inspired. The abnormal cough, however, is not associated with mucus production but may be due to an abnormality of the cough reflex often associated with inflammatory and/or pro-inflammatory conditions of the airway (McEwan et al., 1990). Thus, mechanisms of production and regulation of coughing seem not to be uniform. Foreign substances inspired into the airway should be deposited in regions from the upper airway, such as the nose, to the lower airway, such as the bronchi, depending on the particle size of the substances, while irritating gases and aerosols inspired should be conveyed to the lower airway. With respect to this, Widdicombe (1954) has described that the mechano-receptors for cough production are mainly distributed in the upper trachea and the chemo-receptors in the lower trachea, in particular, around the region of bifurcation of the trachea. These findings seem to indicate that there may be different mechanisms for cough production in different sites of the airway. In turn, coughs triggered in the different sites in the trachea may possibly have different pharmacological properties. In fact, dry irritating coughs are often difficult to treat with antitussives (McEwan et al., 1990; Fuchikami et al., 1990). Recently, Stockwell et al. (1993) reported that superior laryngeal nerve afferents do not play an essential role in the initiation of citric acid-induced cough in humans. In any event, little is known about 'site dependence' of the pharmacological properties of cough.

We now studied the difference in the effects of codeine on cough caused by stimulation to the larynx or to the bifurcation of the trachea of guinea pigs. Because it is difficult to apply chemical stimulation to restricted regions of the trachea, mechanical stimulation was employed.

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2. Materials and methods

2.1. Animals

We used 102 guinea pigs (Male Hartley, 5–7 weeks old). The animals were purchased from Kyudo Pharm (Kumamoto, Japan) and housed in the animal house of the Faculty of Pharmaceutical Sciences, Kumamoto University, at a room temperature of 22 ± 2°C. To prepare the capsaicin-treated animals, a method described previously (Maggi et al., 1987) was followed.

2.2. Cough experiment

Groups consisting of 7–9 animals each were used for a single dose of codeine and vehicle. Guinea pigs were anaesthetized with pentobarbital Na (30 mg/kg, i.p.), because of deep anaesthesia effects on cough reflexes. This level of anaesthesia made it possible to carry out the surgery mentioned below without evoking nociceptive reflexes. The animals were placed on their backs. The anterior skin of the neck was shaved and then cut open in a circle (diameter about 8 mm); the musculature under the skin was also cut open along with the median line to expose the trachea; the portion of the anterior wall of the trachea about 3.5 cm caudal from the cricoid cartilage was cut off in a rectangle (3×4 mm). The operation was very carefully performed to minimize bleeding from the musculature and the tracheal wall. The small hole in the trachea was tightly closed with a small piece of cotton wetted with saline to prevent deterioration of the airways due to drying, except for the time for stimulation.

A schematic diagram of the experimental set-up is shown in Fig. 1. Coughing was monitored and recorded on a polygraph system (RM 6100, Nihon Kohden) through a flowmeter (TUR-3200, Nihon Kohden, Japan) as changes in air flow within a tube connected to a pneumograph placed on to the caudal end of the breast. To calibrate the amplitude of cough response, a ventilation volume amplifier (AQ-601G, Nihon Kohden), connected to the flowmeter, was used. Mechanical stimulation was applied to both sites of the larynx and to the bifurcation of trachea by using a rabbit whisker with the tip 70 μm in diameter. The whisker was inserted through a small hole about 3 cm from the edge of the hole to stimulate both sites (Fig. 1A). After the tip of the stimulator reached the site for stimulation, the stimulator was left for 1 s and carefully drawn out through the hole. Two periods of stimulation were given at 20-min intervals to both sites. The mean amplitude of cough response was taken as a preadministration control value. Then, the effects of the drug were determined: stimulation was applied to both sites at 15, 30, 45 and 60 min after administration of codeine or vehicle, and the amplitude of the response was determined. The value at the most effective time was considered as the post-administration value and expressed as % of the preadministration value. To evaluate the antitussive activity, the mean % of the amplitude was compared for the codeine- and the vehicle-treated groups by means of an unpaired Student's *t*-test. The difference was considered statistically significant, when $P < 0.05$. Codeine (Sankyo, Japan) was diluted in saline and given orally via a gastric tube.

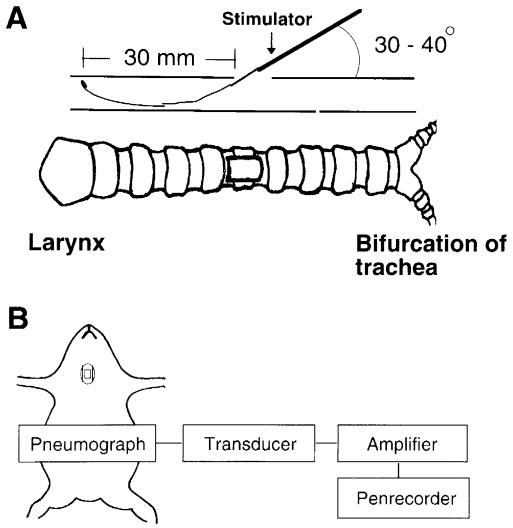

Fig. 1. Method of mechanical stimulation to the larynx and the bifurcation of the trachea. (A) A stimulator, made of a rabbit whisker with tip 70 μm in diameter, was inserted into the larynx or the bifurcation of the trachea, as shown in this figure. (B) A block diagram of the method for recording respiration and cough response.

3. Results

3.1. Stability of cough response

When mechanical stimulation was applied for 1 s to the larynx or to the bifurcation of the trachea, a cough response consisting of a large inspiration and expiration occurred (Fig. 2). The amplitude of the response from either region did not decrease even when stimulation was applied for less than 1 s, indicating that the response was supramaximal. As shown in Fig. 2, there was no significant difference between the amplitude of the responses to larynx or to tracheal bifurcation stimulation. When the amplitude of the control response was taken as 100%, the amplitude of the responses induced at 30, 45 and 60 min after saline administration was as follows: for larynx stimulation, $95.0 \pm 2.1\%$, $95.0 \pm 3.3\%$ and $89.5 \pm 2.3\%$ of the control, respectively; for tracheal bifurcation stimulation, $91.9 \pm 1.7\%$, $93.3 \pm 2.7\%$ and $87.0 \pm 2.55\%$ of the control, respectively. Thus, cough responses caused by mechanical stimulation were stable and reproducible for at least 60 min.

3.2. Effect of codeine in normal guinea pigs

The summarized data are shown in Fig. 3. Codeine, 10, 20 and 50 mg/kg, significantly depressed the cough caused

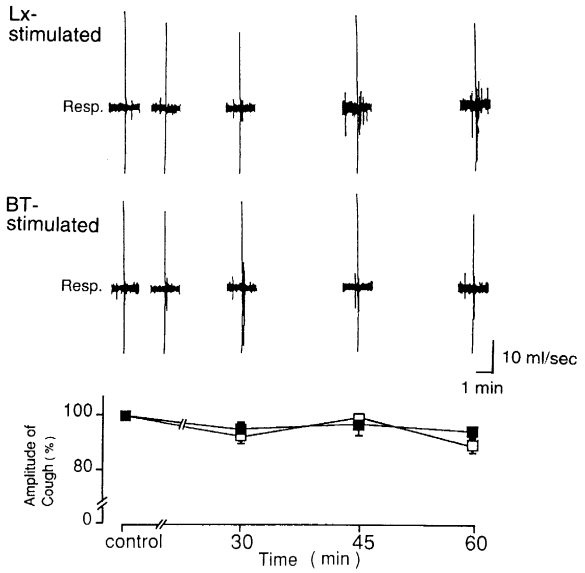

Fig. 2. Typical recording and stability of the cough responses induced by mechanical stimulation to the larynx and the bifurcation of the trachea in guinea-pigs. Upper panel: Upward deflection in each recording represents expiration and downward deflection, inspiration. Lx, larynx; BT, bifurcation of trachea; Resp., respiration. Lower panel: Each value shows the mean ± S.E. ■: larynx stimulation; □: tracheal bifurcation stimulation.

by mechanical stimulation to the larynx. The antitussive, on the other hand, failed to depress the cough caused by stimulation to the tracheal bifurcation, although a large dose of 50 mg/kg significantly depressed the cough. There was a significant difference between the magnitudes of the antitussive effects on the coughs caused by mechanical stimulation to the two sites.

3.3. Effect of codeine in capsaicin-treated guinea pigs

The antitussive effects of 20 mg/kg codeine in normal and in capsaicin-treated guinea pigs were compared. The

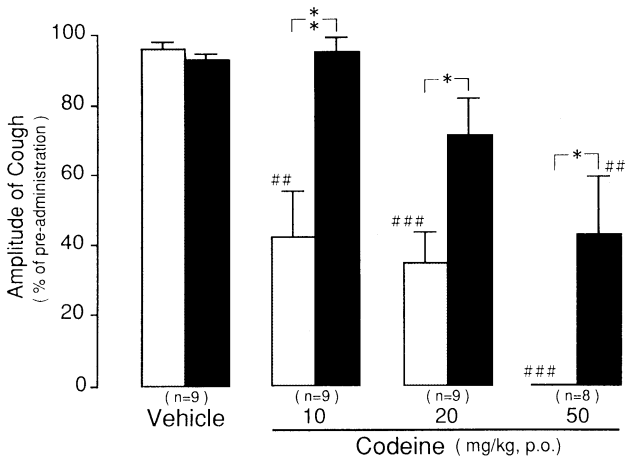

Fig. 3. Comparison of the effects of codeine on cough response induced by mechanical stimulation to the larynx (open columns) and the bifurcation of the trachea (filled columns) in normal guinea-pigs. Each value shows the mean ± S.E. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from the corresponding group of larynx stimulation. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, significantly different from the vehicle.

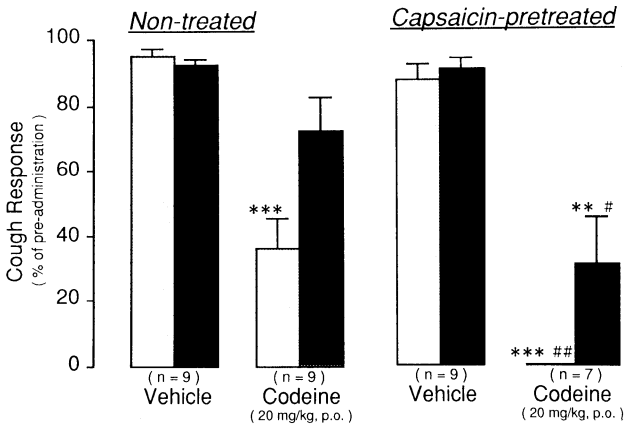

Fig. 4. The effects of codeine on the cough response induced by mechanical stimulation to the larynx (open columns) and to the bifurcation of the trachea (filled columns) in capsaicin-treated guinea-pigs. Each value shows the mean ± S.E. $^{**}P < 0.01$, $^{***}P < 0.001$, significantly different from the vehicle. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, significantly different from the corresponding non-treated group.

amplitude and the reproducibility of cough responses caused by mechanical stimulation to the larynx or the bifurcation of trachea were comparable between the two groups of animals (Fig. 4). As shown in Fig. 4, codeine, 20 mg/kg, abolished the cough caused by stimulation to the larynx in capsaicin-treated animals, although the same dose of codeine did not do so in the normal animals (Fig. 4). In addition, 20 mg/kg codeine significantly depressed the amplitude of cough caused by stimulation to the bifurcation in capsaicin-treated animals, whereas no significant depression was observed in non-treated animals (Fig. 4).

4. Discussion

This is the first demonstration that an antitussive, codeine, differentially depressed the cough caused by mechanical stimulation to the larynx and that caused by stimulation to the bifurcation of the trachea in guinea pigs. Mechanical stimulation to both the larynx and the bifurcation of the trachea both caused a stable cough response over 60 min, when applied at 15 min intervals. It is unlikely that the response obtained at the bifurcation was supramaximal and the response at the larynx was clearly not, because (1) the amplitude of the responses to stimulation to the larynx and to the tracheal bifurcation was comparable and (2) the amplitude of the response at either region did not decrease, even when a weak stimulus was applied. It is, therefore, unlikely that the differential effect of codeine was due to experimental artifacts.

Widdicombe (1989) has reported that in cats, the larynx was the most sensitive part of the airway to cough induced by mechanical stimulation. A recent study has shown that mechanical and chemical stimulation to the larynx produced cough in 56.7% and 33.3% of dogs studied, respec-

tively, while when applied to the tracheal bifurcation, the two types of stimulation caused cough in all animals studied (Tatar et al., 1994). No report, however, is available about differences in cough sensitivity between the two regions of the airways in guinea pigs, although preliminary work has shown that guinea pigs with the superior laryngeal nerves cut coughed more in response to citric acid and nicotine than did controls, suggesting the existence of an inhibitory component of the superior laryngeal nerves (Forsberg et al., 1990). It seems, however, unlikely that, in guinea pigs, the larynx was less insensitive to cough production by mechanical stimulation than was the bifurcation of the trachea, because no significant difference in the intensity of cough responses was found between the two regions, when the amplitude of the responses was taken as a parameter and observed over 60 min. Therefore, the present results suggests that the cough induced by stimulation to the bifurcation of trachea might be more resistant to codeine treatment than that caused by larynx stimulation.

Codeine 20 mg/kg did not completely depress the cough responses caused by mechanical stimulation to the larynx in normal guinea pigs, but abolished the response in capsaicin-treated animals. Large doses of capsaicin cause degeneration and dysfunction of the C-fibres (Jancso et al., 1977; Lundberg and Saria, 1983; Maggi et al., 1987), and block the cough caused by citric acid and capsaicin, but not that caused by nicotine and mechanical stimulation (Forsberg et al., 1988). On the other hand, Jancso (1992) has reported that capsaicin treatment destroys not only C-fibres but also Aδ-myelinated fibres. Further, Widdicombe (1995) has described that C-fibre receptors may be involved in coughing by releasing sensory neuropeptides which, in turn, act on rapidly adapting receptors. If Aδ-fibre receptors are the only type of receptors for directly stimulating cough, codeine should depress equally cough responses in normal and in capsaicin-treated animals to mechanical stimulation to the larynx. However, we found a significant difference in the antitussive effects of codeine between two groups of animals. It may still be possible that the codeine-resistant component of the cough might be induced through the action of sensory neuropeptides released by activation of C-fibres, although it remains to be studied whether or not activation of the C-fibres directly stimulates coughing. Further, the results also suggest that cough responses caused by stimulation to the larynx in normal guinea pigs might consist of at least two components: one, a codeine-resistant component in which sensory neuropeptides are at least partly involved, and the other, a codeine-sensitive component in which the neuropeptides are not involved. In contrast to the cough caused by larynx stimulation, the cough caused by stimulation to the bifurcation of the trachea was not completely abolished by codeine, 20 mg/kg. It was not determined whether part of the cough caused by stimulation to the bifurcation is completely insensitive to codeine or not. However, our preliminary study showed that codeine at the very high dose of 100 mg/kg did not abolish the response in normal guinea pigs. It seems likely that the cough caused by stimulation to the bifurcation of trachea might consist of at least the two components described above. Further, the results seem to suggest that there might be some difference in the ratio of the two components for coughs induced by stimulation of the larynx and those induced by stimulation to the bifurcation of the trachea.

The amplitude of cough responses in capsaicin-treated animals was comparable to that in normal guinea pigs. Possible explanations for this are the following: the intensity of mechanical stimulation might have been supra-maximal; mechanical stimulation stimulated both A-fibres and C-fibres; the intensity of mechanical stimulation was strong enough to induce full cough responses in capsaicin-treated animals. Although, in normal guinea pigs, cough responses caused by stimulation to the bifurcation of the trachea were slightly depressed by 20 mg/kg codeine, the depression was not statistically significant. The same dose of codeine, however, depressed significantly the responses in capsaicin-treated animals. This result suggests that a codeine-sensitive component might be predominant in the cough caused by mechanical stimulation to the bifurcation of the trachea of capsaicin-treated animals.

Capsaicin-sensitive sensory nerves in guinea-pig airways, which are believed to be the C-fibres, have been characterized as peptidergic fibres containing both tachykinins and calcitonin gene-related peptide (Martling, 1987). In addition, it has been reported that the density of capsaicin-sensitive afferent fibres varies in different tracts of the respiratory system (Lundberg et al., 1983; Bucsics et al., 1983; Cadieux et al., 1986; Palmer et al., 1987). Manzini et al. (1989) revealed that the substance P-like immunoreactivity levels and its release by capsaicin in bronchial tissues were about five to eight times greater than those in tracheal tissues, suggesting a higher number of capsaicin-sensitive peptidergic fibres and/or a greater neuropeptide content per nerve ending in the bronchus than in the trachea. Our preliminary histo-chemical study also revealed that regions around the larynx showed a lower substance P-like immunoreactivity than did the region of the tracheal bifurcation in guinea pigs. These findings supports the above contention.

Recently, Stockwell et al. (1993) reported that afferents of the superior laryngeal nerves do not play an essential role in the initiation of citric acid-induced cough in humans. This report seems to be in agreement with the present finding that the C-fibre (sensory neuropeptides)-mediated component was not predominant in the cough caused by larynx stimulation, because (1) capsaicin is well known to stimulate the C-fibres (Jancso et al., 1977); (2) citric acid should act as acid on the afferent nerves for cough production; (3) a capsaicin-activated current was also mimicked by acidic stimuli, suggesting that capsaicin and acid may activate the same receptor (Liu and Simon, 1994).

There are two possible interpretations for the mechanisms of the differential effect of codeine: (1) the afferent fibres directly activated by mechanical stimulation and those activated by sensory neuropeptides may not converge into the common central pathway in the medulla oblongata; (2) the μ- and κ-opioid receptors may be distributed mainly in the upper airway. With respect to the first possibility, it has been shown that part of the afferent pathways arising from the lower airway of guinea pigs project directly into the spinal cord (Kummer et al., 1992), although it is unknown whether or not this pathway is involved in cough reflexes. With respect to the second possibility, Karlsson et al. (1989) have reported that opioids depress cough and reflex bronchoconstriction through an action on μ- and κ-opioid receptors located in the tracheobronchial tree of guinea pigs. However, little is known about the regional distribution of the opioid receptors in the tracheobronchial tree. It is still difficult to reach conclusions about the mechanism of the codeine-resistant component of cough in guinea-pigs. Further studies are needed to clarify this issue.

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