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# Inhibitory Effects of Non-narcotic Antitussive Drugs on Cholinergically and Non-cholinergically Mediated Neurogenic Contractions of Guinea-pig Isolated Bronchial Muscle

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**Abstract**—We have examined the actions of non-narcotic antitussive drugs on the response evoked by electrical field stimulation or by acetylcholine (ACh) and neurokinin A (NKA) on guinea-pig bronchial strip chain. Electrical field stimulation (1–32 Hz, 0·5 ms, 30 V for 5 s) evoked a biphasic contraction in a frequency-dependent manner, consisting of a cholinergically mediated fast contraction followed by a non-cholinergically mediated slow contraction. Dextromethorphan (1–300 μM) and tipepidine (0·1–100 μM) caused a concentration-dependent inhibition in the height of the biphasic contraction, but noscapine (1–300 μM) was less effective. Submaximal contractions of bronchial muscle evoked by exogenous ACh (1–30 μM) were inhibited by tipepidine (10–100 μM), but not by dextromethorphan (10–100 μM) or noscapine (10–100 μM), while those evoked by exogenous NKA (10–300 nM) were augmented by these drugs. The results indicate that in guinea-pig isolated bronchial muscle, dextromethorphan inhibited both neurally-mediated responses but not those to the exogenously applied agent. Tipepidine caused an inhibition similar to the non-cholinergically mediated response of dextromethorphan, it also caused a more profound inhibition of the cholinergically mediated response and selectively antagonized ACh. Noscapine had no effect.

Recently, we have reported that catecholamines, purine compounds and opioid agonists can inhibit excitatory cholinergic and non-cholinergic neurotransmission of the guinea-pig airways via prejunctional $\alpha_2$-adrenoceptors, $P_1$-purinoceptors and $\kappa$-opioid receptors, respectively (Kamikawa & Shimo 1986, 1989, 1990 a, b). Similar observations were made by others (Grundström et al 1984; Frossard & Barnes 1987). Recent evidence indicates that the site of the antitussive actions of morphine and codeine may be not only the cough centre in the medulla oblongata but also peripheral nerve endings in the lung (Kasé 1980; Yanaura et al 1981; Parsons et al 1986; Karlsson et al 1988a). BW443C, a newly synthesized enkephalin analogue with poor penetration into the brain, had a peripheral rather than a central antitussive action in the guinea-pig (Adcock et al 1988), and inhibited non-cholinergic neurally mediated contraction of the guinea-pig isolated bronchial muscle (Shankley et al 1989; Kamikawa & Shimo 1990b). Non-cholinergic bronchoconstrictions are thought to be mediated by the release of substance P or neurokinin A (NKA) from pulmonary sensory nerve endings (Andersson & Grundström 1987; Lundberg & Saria 1987). Since sensory nerves in the airways have a potential role for producing cough and bronchoconstriction as a defense reflex (Karlsson et al 1988b; Coleridge et al 1989), these findings suggest that non-cholinergic neurotransmission may be a peripheral target for antitussive drugs. Hence, in the present study we have investigated the modulating effects of non-narcotic antitussive drugs on electrically induced neurogenic contractions of the guinea-pig bronchi. A preliminary report of some of these results has been made (Kamikawa & Shimo 1990c).

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## Materials and Methods

Male guinea-pigs, 300–700 g, were anaesthetized with diethyl ether, and bled from the cervical artery. The tracheobronchial tree was excised and the bronchial strip chain was prepared (Kamikawa & Shimo 1989). Briefly, two pieces of right and left bronchial transverse strips, 2–3 mm wide, were connected in alignment with threads and immersed in a 10 mL organ bath filled with modified Krebs bicarbonate solution of the following composition (mM); NaCl 120, KCl 4·7, $CaCl_2$ 2·5, $MgCl_2$ 1·2, $NaHCO_3$ 25, $KH_2PO_4$ 1·2, disodium edetate 0·03, ascorbic acid 0·12 and glucose 11 (pH 7·4). The Krebs solution always contained 20 μM choline chloride and was bubbled with 5% carbon dioxide in oxygen, and maintained at 37°C.

The preparation was suspended under an initial tension of 0·5 g and 60 min was allowed to elapse before experiments were started. The bronchial response was isometrically recorded by means of a force-displacement transducer (Nihon Kohden SB-IT-H) and a Nihon Kohden polygraph recorder (RJG-4004). Electrical field stimulation was with rectangular pulses of 1–32 Hz frequency, 0·5 ms duration and supramaximal voltage for 5 s, through bipolar platinum electrodes which were 10 mm apart and connected to a Nihon Kohden stimulator (SEN-1101). For the elimination of endogenous prostaglandin biosynthesis in response to field stimulation, the Krebs solution contained 2 μM indomethacin. When the strip was electrically stimulated, a biphasic contraction was obtained at every stimulus frequency. The response was composed of an initial fast contraction followed by a sustained contraction which was mediated by cholinergic and non-cholinergic nerve stimulation, respectively (Kamikawa & Shimo 1989). The heights of these contractions were comparable with those of submaximal contractions evoked by exogenous acetylcholine (ACh,

1–30 μM) and NKA (10–300 nM), respectively. The effects of non-narcotic antitussive drugs on electrically induced contractions were measured as the percentage changes from the original contraction height just before the drug was applied to the bath. Data are expressed as the mean ± s.e.m. Each experimental group consisted of 6–11 preparations taken from different animals. Student's *t*-test for paired or unpaired observations was used for statistical evaluation of the data. $P < 0.05$ was considered significant.

Drugs used were dextromethorphan hydrobromide (Shionogi), noscapine hydrochloride (Sigma), tipepidine dibenzoate (Tanabe), naloxone hydrochloride (Endo), yohimbine hydrochloride (Sigma), propranolol hydrochloride (Sigma), aminophylline (Sigma), indomethacin (Sankyo), acetylcholine chloride (Daiichi) and neurokinin A (Peptide Institute). To prepare the drug solutions, indomethacin was dissolved in distilled water containing equimolar concentrations of $Na_2CO_3$ and diluted with 0·9% w/v NaCl (saline); all other drugs were dissolved in and diluted with saline. The molar concentrations of drugs in this paper refer to the final bath concentrations.

## Results

*Effect of dextromethorphan*

When the bronchial strip chain was electrically stimulated at various frequencies from 1 to 32 Hz, a biphasic contraction was obtained in a frequency-dependent manner (Fig. 1A). After the pretreatment with dextromethorphan (100 μM), the biphasic contraction was markedly inhibited at all stimulus frequencies with a progressive decline in resting tone (Fig. 1B). The response was concentration-dependent and reversible by washing. Fig. 2A summarizes the inhibitory effect of dextromethorphan on the cholinergic and non-cholinergic components of the electrically-induced contraction. Both components at every stimulus frequency were equally inhibited by the pretreatment with 10 μM dextromethorphan, and were abolished with 100 μM. When the bronchial muscle was repetitively stimulated at 8 Hz every 20 min, reproducible biphasic contractions were obtained. Dextromethorphan, at

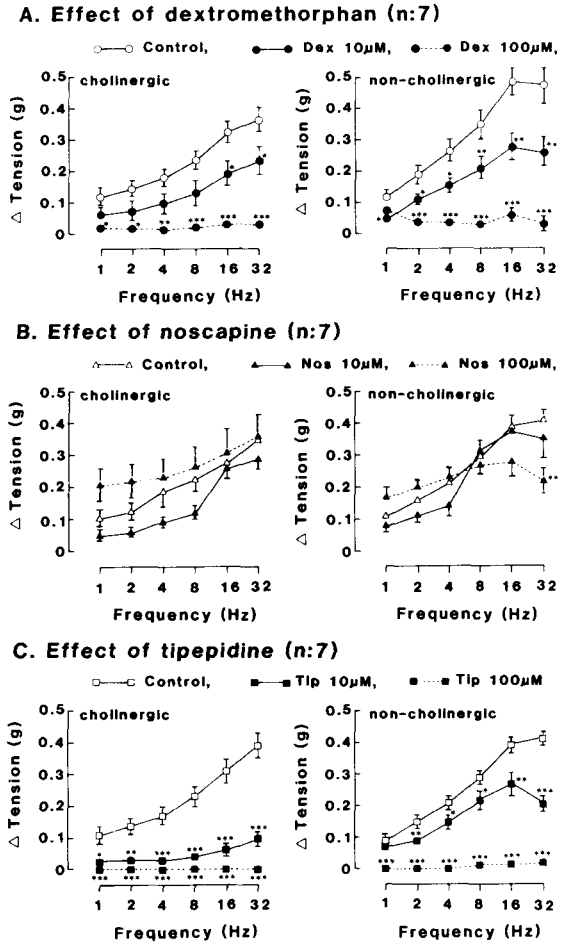


FIG. 2. Frequency-response relationship for biphasic contraction to electrical field stimulation (1–32 Hz, 0·5 ms, 30 V for 5 s) of guinea-pig bronchial strip chain preparation in the absence (open symbols) or presence (closed symbols) of 10 μM (solid lines) and 100 μM (dotted lines) of non-narcotic antitussive drugs. Left panels, cholinergically mediated fast contraction; right panels, non-cholinergically mediated sustained contraction. Note that dextromethorphan and tipepidine significantly inhibited both neurogenic contractions. Ordinates, tension development from resting tone. Each point represents mean ± s.e.m. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

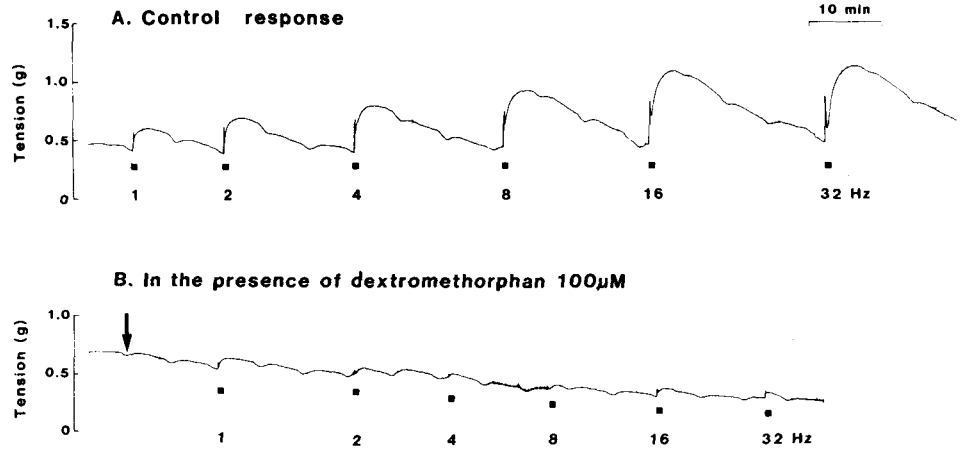


FIG. 1. Representative trace of biphasic contraction to electrical field stimulation (1–32 Hz, 0·5 ms, 30 V for 5 s at ■) of guinea-pig bronchial strip chain preparation before (A) and after (B) administration of dextromethorphan (100 μM at arrow). Note that dextromethorphan reduced amplitude of electrically induced biphasic contraction at every stimulus frequency.

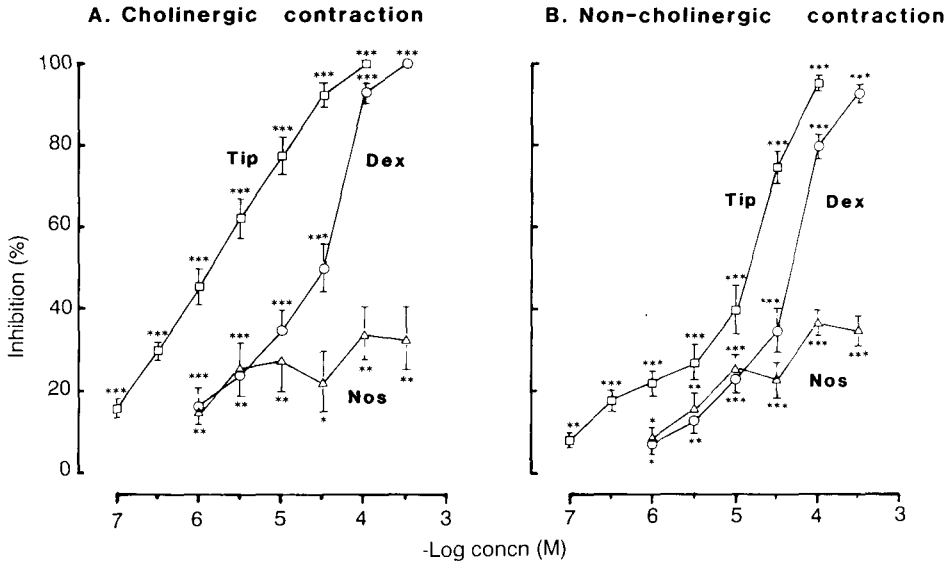


FIG. 3. Cumulative log concentration-response curves for the inhibitory actions of non-narcotic antitussive drugs on electrically (8 Hz, 0·5 ms, 30 V for 5 s) induced cholinergic (A) and non-cholinergic (B) contractions of guinea-pig bronchial strip chain preparation. Each point represents mean ± s.e.m. Tip, tipepidine; Dex, dextromethorphan; Nos, noscapine. Numbers of observations are shown in Table 1. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 1. Inhibitory activities of the non-narcotic antitussive drugs on cholinergically and non-cholinergically mediated neurogenic contractions of guinea-pig bronchial strip chain preparations evoked by 8 Hz electrical stimulation.

| | | $-\text{Log}_{10}$ IC50 | |
|---|---|---|---|
| Antitussive drug | n | Cholinergic | Non-cholinergic |
| Dextromethorphan | 9 | 4·60 ± 0·10 | 4·31 ± 0·07* |
| Noscapine | 10 | < 3·50 | < 3·50 |
| Tipepidine | 11 | 5·81 ± 0·12 | 4·92 ± 0·07*** |

All values represent the mean ± s.e.m. of the −log molar concentrations causing 50% reduction of the contraction height. * $P<0.05$; *** $P<0.001$. These were compared with the value for cholinergic component using the unpaired $t$-test. The values of tipepidine for cholinergic and noncholinergic components were also significantly greater than those of dextromethorphan ($P<0.001$).

higher concentrations than 1 μM, inhibited the response in a concentration-dependent manner (Fig. 3). The concentration of dextromethorphan required to inhibit the contraction height by 50% ($-\log_{10}$IC50, mean ± s.e.m.) was 4·60 ± 0·10 for the cholinergic component and 4·31 ± 0·07 for the non-cholinergic component (Table 1). At the concentrations examined (10 and 100 μM), dextromethorphan did not modify submaximal contractions of bronchial muscle evoked by exogenous ACh (1–30 μM) (Fig. 4A). Submaximal contractions evoked by NKA (10–300 nM) were augmented by the pretreatment with 10 μM, but not 100 μM, dextromethorphan.

*Effect of noscapine*

Noscapine (10 and 100 μM) caused no significant change to the biphasic contraction evoked by stimulation (1–16 Hz) (Fig. 2B). Only the non-cholinergically mediated contraction evoked by a high frequency stimulation (32 Hz) was significantly inhibited by 100 μM noscapine. The biphasic contraction evoked by 8 Hz electrical stimulation was inhibited only 10–30% by noscapine at a concentration ranging from 1 to 300 μM (Fig. 3). Submaximal contractions of bronchial muscle evoked by exogenous ACh (1–30 μM) or NKA (10–300 nM) were not significantly modified by the pretreatment with 10 or 100 μM noscapine (Fig. 4B).

*Effect of tipepidine*

Tipepidine (10 μM) significantly inhibited the electrically induced biphasic contraction at every stimulus frequency and the cholinergic component was more inhibited than the non-cholinergic component (Fig. 2C). Tipepidine (100 μM) abolished the response at all stimulus frequencies. Tipepidine, at higher concentrations than 0·1 μM, inhibited the biphasic contraction evoked by 8 Hz electrical stimulation in a concentration-dependent manner (Fig. 3). The IC50 value was 5·81 ± 0·12 for the cholinergic component and 4·92 ± 0·07 for the non-cholinergic component (Table 1). Submaximal contractions of bronchial muscle evoked by exogenous ACh (1–30 μM) were concentration-dependently inhibited by tipepidine (10–100 μM), while those by exogenous NKA (10–300 nM) were augmented by 10 μM tipepidine (Fig. 4C).

*Effect of receptor antagonists*

The inhibitory action of antitussive drugs on the biphasic contraction to 8 Hz electrical stimulation were examined under conditions of pre-treatment or post-treatment with various receptor antagonists. Yohimbine (2 μM, n = 9), propranolol (2 μM, n = 6), naloxone (10 μM, n = 9) and aminophylline (10 μM, n = 9) were unable to reverse the inhibitory actions of dextromethorphan (100 μM), noscapine (100 μM) and tipepidine (100 μM).

## Discussion

In this study we have demonstrated that dextromethorphan, noscapine and tipepidine inhibit the electrically induced neurogenic contractions of guinea-pig bronchial muscle. The

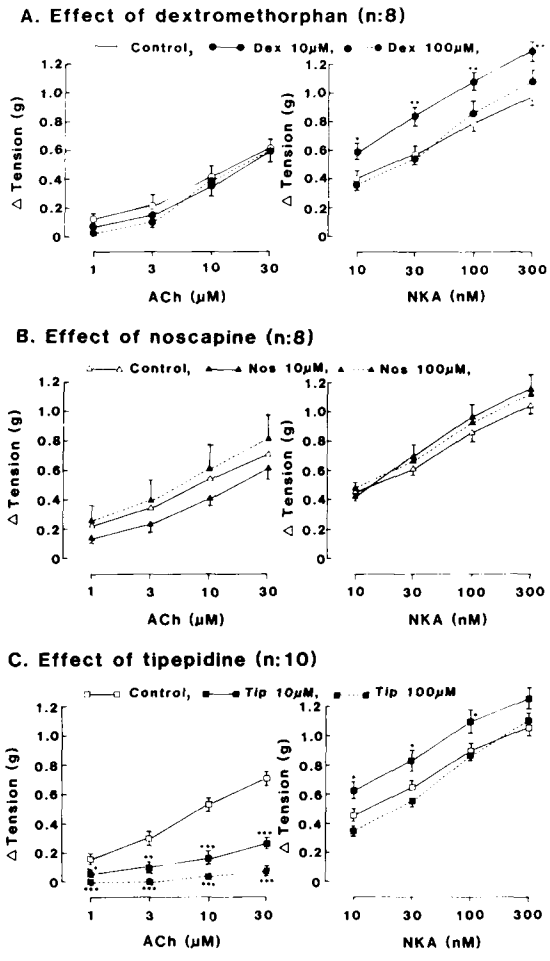


FIG. 4. Cumulative log concentration-response curves for submaximal contractions to exogenous acetylcholine (ACh, left panels) and neurokinin A (NKA, right panels) of guinea-pig bronchial strip chain preparation in the absence (open symbols) or presence (closed symbols) of 10 μM (solid lines) and 100 μM (dotted lines) of non-narcotic antitussive drugs. The ordinates show the developed tension by ACh or NKA. Each point represents mean ± s.e.m. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

order of inhibitory activity was tipepidine > dextromethorphan ≫ noscapine. Dextromethorphan and tipepidine more effectively inhibited the cholinergic component than the non-cholinergic component of the electrically induced response. Submaximal contraction to exogenous ACh was unaffected by dextromethorphan or noscapine, but was inhibited by tipepidine. Dextromethorphan and tipepidine augmented submaximal contraction to exogenous NKA. These data suggest that the inhibitory actions of dextromethorphan and noscapine on neurogenic contractions are mediated solely by a pre-junctional reduction of the transmitter release from cholinergic and non-cholinergic nerves. The inhibitory action of tipepidine seems to be mediated by a post-junctional inhibition of cholinoceptors and a pre-junctional reduction of the transmitter release from non-cholinergic nerves. The pre-junctional inhibitory actions of these drugs are thought not to be mediated by $\alpha_2$- and $\beta$-adrenoceptors, opioid receptors or purinoceptors, because the actions were not antagonized by yohimbine, propranolol, naloxone and aminophylline.

It has been reported that narcotic antitussive drugs can selectively inhibit non-cholinergic bronchoconstriction via pre-junctional opioid receptors (Frossard & Barnes 1987; Buchan et al 1989; Shankley et al 1989; Kamikawa & Shimo 1990b). Although antitussive drugs are generally thought to exert their action by suppressing the cough centre in the medulla (Kasé 1980), recent evidence suggests that narcotic antitussive drugs also have a peripheral site of action in the lung (Yanaura et al 1981; Parsons et al 1986; Adcock et al 1988; Karlsson et al 1988a). As a possible site of action of the narcotics, non-cholinergic nerves or sensory nerves in the airways have been given attention (Adcock et al 1987; Shankley et al 1989; Buchan et al 1989). Much evidence indicates that non-cholinergic bronchoconstriction is mediated by the release of substance P or NKA from sensory nerve endings (Lundberg et al 1983; Andersson & Grundström 1987; Kamikawa & Shimo 1989). The present study provided further evidence supporting the theory that antitussive drugs may exert their pharmacological actions by inhibiting the tachykinin-containing sensory nerve endings. The idea is also supported by the fact that cough and bronchoconstriction are concurrently evoked by the stimulation of sensory nerve endings in the airways as a defense reflex (Salem & Aviado 1964; Karlsson et al 1988b).

The exact receptor mechanism of non-narcotic antitussive drugs for inhibiting non-cholinergic neurotransmission could not be determined from the present results. Previously, Craviso & Musacchio (1983a) have reported that dextromethorphan has its specific binding site in the guinea-pig brain. They have also found that other antitussive drugs such as caramiphen and carbetapentane display nanomolar affinity for this binding site (Craviso & Musacchio 1983b), suggesting that the dextromethorphan binding site mediates the antitussive action. Since phenytoin and noscapine produced an allosteric enhancement of the binding of dextromethorphan, these authors suspected that antitussive drugs may have anticonvulsant properties. This was confirmed by the finding that dextromethorphan and carbetapentane protect rats against maximal electroshock seizure and enhance the anticonvulsant effect of phenytoin (Tortella & Musacchio 1986). We also had reported that tipepidine has a specific anticonvulsant activity on tonic extension of mice elicited by maximal electroshock, in addition to its antitussive action (Kasé et al 1959, 1970). Together with these findings, the present results suggest that non-narcotic antitussive drugs may inhibit non-cholinergic neurotransmission via a common receptor mechanism.

In conclusion, dextromethorphan inhibited neurally-mediated responses but not those to the exogenously applied agents of guinea-pig bronchial muscle. Tipepidine caused similar inhibition to dextromethorphan of the non-cholinergically mediated response, a more profound inhibition of the cholinergically mediated one and selectively antagonized ACh. Noscapine had little effect.

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## References


Adcock, J. J., Allan, G., Richardson, P. J., Schneider, C., Smith, T. W. (1987) Antitussive effects and inhibition of vagal sensory nerve activity by Tyr.D.Arg.Gly.Phe(4-$NO_2$).Pro.$NH_2$ (443C). Br. J. Pharmacol. 90: 143P

Adcock, J. J., Schneider, C., Smith, T. W. (1988) Effects of codeine, morphine and a novel opioid pentapeptide BW443C, on cough, nociception and ventilation in the unanaesthetized guinea-pig. Ibid. 93: 93–100

Andersson, R. G. G., Grundström, N. (1987) Innervation of airway smooth muscle. Efferent mechanisms. Pharmacol. Ther. 32: 107–130

Buchan, P., Smith, T. W., Adcock, J. J. (1989) The effect of BW443C and codeine on capsaicin-induced bronchoconstriction in the guinea-pig. Br. J. Pharmacol. 96: 250P

Coleridge, H. M., Coleridge, J. C. G., Schultz, H.D. (1989) Afferent pathways involved in reflex regulation of airway smooth muscle. Pharmacol. Ther. 42: 1–63

Craviso, G. L., Musacchio, J. M. (1983a) High-affinity dextromethorphan binding sites in guinea-pig brain. I. Initial characterization. Mol. Pharmacol. 23: 619–628

Craviso, G. L., Musacchio, J. M. (1983b) High-affinity dextromethorphan binding sites in guinea-pig brain. II. Competition experiments. Ibid. 23: 629–640

Frossard, N., Barnes, P. J. (1987) $\mu$-Opioid receptors modulate non-cholinergic nerves in guinea-pig airways. Eur. J. Pharmacol. 141: 519–522

Grundström, N., Andersson, R. G. G., Wikberg, J. E. S. (1984) Inhibition of the excitatory non-adrenergic, non-cholinergic neurotransmission in the guinea-pig tracheo-bronchial tree mediated by $\alpha_2$-adrenoceptors. Acta Pharmacol. Toxicol. 54: 8–14

Kamikawa, Y., Shimo, Y. (1986) Inhibitory effects of sympathomimetic drugs on cholinergically mediated contractions of guinea-pig isolated tracheal muscle. J. Pharm. Pharmacol. 38: 742–747

Kamikawa, Y., Shimo, Y. (1989) Adenosine selectively inhibits non-cholinergic transmission in guinea pig bronchi. J. Appl. Physiol. 66: 2084–2091

Kamikawa, Y., Shimo, Y. (1990a) Inhibitory effects of catecholamines on cholinergically and non-cholinergically mediated contractions of guinea-pig isolated bronchial muscle. J. Pharm. Pharmacol. 42: 131–134

Kamikawa, Y., Shimo, Y. (1990b) Morphine and opioid peptides selectively inhibit the non-cholinergically mediated neurogenic contraction of guinea-pig isolated bronchial muscle. Ibid. 42: 214–216

Kamikawa, Y., Shimo, Y. (1990c) Inhibitory effect of non-narcotic antitussive drugs on neurogenic contractions of the guinea-pig isolated bronchial muscle. Jap. J. Pharmacol. 52(Suppl. I): 186p

Karlsson, J.-A., Lanner, A.-S., Forsberg, K., Fuller, R. W., Persson, C. G. A. (1988a) Inhibition of cough and bronchoconstriction in the guinea-pig by opiates: evidence for a peripheral action. Br. J. Pharmacol. 93: 233P

Karlsson, J.-A., Sant 'Ambrogio, G., Widdicombe, J. (1988b) Afferent neural pathways in cough and reflex bronchoconstriction. J. Appl. Physiol. 65: 1007–1023

Kasé, Y. (1980) Antitussive agents and their sites of action. Trends Pharmacol. Sci. 1: 237–239

Kasé, Y., Kamikawa, Y., Kuramoto, I. (1970) Anticonvulsant activity of 1-methyl-3-di(thien-2-yl)methylenepiperidine. Folia Pharmacol. Jpn. 66: 31§ (abstract in Japanese)

Kasé, Y., Yuizono, T., Yamasaki, T., Yamada, T., Io, S., Tamiya, M., Kondo, I. (1959) A new potent non-narcotic antitussive, 1-methyl-3-di-(2'-thienyl)-methylene piperidine. Pharmacology and clinical efficacy. Chem. Pharm. Bull. 7: 372–377

Lundberg, J. M., Saria, A. (1987) Polypeptide-containing neurons in airway smooth muscle. Ann. Rev. Physiol. 49: 557–572

Lundberg, J. M., Saria, A., Brodin, E., Rosell, S., Folkers, K. (1983) A substance P antagonist inhibits vagally induced increase in vascular permeability and bronchial smooth muscle contraction in the guinea-pig. Proc. Nat. Acad. Sci. USA 80: 1120–1124

Parsons, D. N., Schneider, C., Smith, T. W. (1986) Peripheral antitussive action of codeine and morphine in the guinea-pig. Br. J. Pharmacol. 89: 818P

Salem, H., Aviado, D. M. (1964) Antitussive drugs with special reference to a new theory for the initiation of the cough reflex and the influence of bronchodilators. Am. J. Med. Sci. 247: 585–600

Shankley, C. E., Anderson, C. W. P., Adcock, J. J. (1989) The effect of BW443C on non-cholinergic bronchoconstriction in the guinea-pig in vitro and in vivo. Br. J. Pharmacol. 96: 249P

Tortella, F. C., Musacchio, J. M. (1986) Dextromethorphan and carbetapentane: centrally acting non-opioid antitussive agents with novel anticonvulsant properties. Brain Res. 383: 314–318

Yanaura, S., Hosokawa, T., Kitagawa, H., Misawa, M. (1981) Peripheral effects of morphine and codeine on the cough reflex. Jap. J. Pharmacol. 31: 529–536