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Antitussive effects of $GABA_B$ agonists in the cat and guinea-pig

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1 $GABA_B$ agonists inhibit neuronal processes which are important in the pathogenesis of airway disease, such as bronchospasm. Cough is a prominent symptom of pulmonary disease, but the effects of $GABA_B$ agonists on this airway reflex are unknown. Experiments were conducted to determine the antitussive effect of $GABA_B$ receptor agonists in comparison to the known antitussive agents, codeine and dextromethorphan.

2 Unanaesthetized guinea-pigs were exposed to aerosols of 0.3 mM capsaicin to elicit coughing, which was detected with a microphone and counted. Cough also was produced in anaesthetized cats by mechanical stimulation of the intrathoracic trachea and was recorded from electromyograms of respiratory muscle activity.

3 In guinea-pigs, the $GABA_B$ agonists baclofen and 3-aminopropyl-phosphinic acid (3-APPi) produced dose-dependent inhibition of capsaicin-induced cough when administered by subcutaneous or inhaled routes. The potencies of baclofen and 3-APPi compared favourably with codeine and dextromethorphan.

4 The $GABA_B$ antagonist, CGP 35348 (0.3– 30 mg kg^{-1}, s.c.) inhibited the antitussive effect of baclofen (3.0 mg kg^{-1}, s.c.). However, CGP 35348 (10 mg kg^{-1}, s.c.) had no effect on the antitussive activity of codeine (30 mg kg^{-1}, s.c.). The antitussive effect of baclofen was not influenced by the $GABA_A$ antagonist, bicuculline (3 mg kg^{-1}, s.c.) or naloxone (0.3 mg kg^{-1}, s.c.).

5 In the cat, baclofen (0.3–3.0 mg kg^{-1}, i.v.) decreased mechanically-induced cough in a dose-dependent manner. In this model, baclofen (ED_{50} = 0.63 mg kg^{-1}) was less potent than either codeine or dextromethorphan. The antitussive effect of baclofen in the cat was antagonized by the $GABA_B$ antagonists, CGP 35348 (10 mg kg^{-1}, i.v.) and 3-aminopropylphosphonic acid (3 mg kg^{-1}, i.v.).

6 We show that baclofen and 3-APPi have antitussive effects in the guinea-pig and cat and these effects are mediated by $GABA_B$ receptors.

Keywords: $GABA_B$ receptors; cough; antitussive; CGP35348; baclofen; 3-aminopropyl-phosphinic acid

Introduction

Cough is a defensive reflex that is often present during pulmonary diseases such as chronic bronchitis, asthma, pulmonary neoplasm, upper respiratory infections, and pulmonary fibrosis (Braman & Corrao, 1987; O'Connell *et al.*, 1991). The function of this reflex is to remove fluids, mucus, and/or foreign bodies from the respiratory tract by the generation of rapid airflows (Korpas & Tomori, 1979). Cough is generally considered to be a beneficial event, but there are situations in which this reflex is associated with significant morbidity. Chronic cough is associated with exacerbation of asthmatic symptoms, rib fractures, breathlessness, ruptured abdominal muscles, pneumothorax, syncope, second and third degree heart block, and loss of consciousness (Braman & Corrao, 1987; O'Connell *et al.*, 1991; Young *et al.*, 1991). Therapy for chronic cough can include administration of antitussive agents, the most prominent of which are codeine and dextromethorphan (Braman & Corrao, 1987). Other agents known to have antitussive effects in animal models include dopamine receptor agonists (Kamei *et al.*, 1987a), N-methyl-D-aspartate antagonists (Kamei *et al.*, 1989) and the peripherally acting opioid agonist BW 443C (Adcock *et al.*, 1988). Furthermore, the use of 5-hydroxytryptamine (5-HT) receptor antagonists and depletion of brain (5-HT) levels have suggested an inhibitory effect of this monoamine on cough (Kamei *et al.*, 1986; Kamei *et al.*, 1987b).

Another inhibitory neurochemical which has been implicated in depression of the cough reflex is γ-aminobutyric acid (GABA) (Nosalova *et al.*, 1987). GABA is present throughout the peripheral and central nervous systems (Mugnaini & Oertel, 1985; Erdo & Kiss, 1986) and binds to at least two different receptors, termed $GABA_A$ and $GABA_B$ (Bowery, 1989). Gabalineamide, an analogue of GABA, has antitussive effects in the cat (Nosalova *et al.*, 1987). It was not known whether this action was due to an effect of $GABA_A$ or $GABA_B$ receptor stimulation. However, there is good evidence that $GABA_B$ receptors inhibit the activity of peripheral sensory afferents (Green & Cottrell, 1988) such as pulmonary C-fibres (Belvisi *et al.*, 1989) that may influence the production of cough (Forsberg & Karlsson, 1986). Therefore, we speculated that $GABA_B$ agonists would have antitussive activity and we studied this phenomenon in several animal models of cough. A preliminary account of this work has been published (Bolser *et al.*, 1991b).

Methods

Irritant-induced cough in guinea-pigs

Unanaesthetized male Dunkin-Hartley guinea-pigs (250–600 g) were placed in a transparent plastic chamber and exposed to aerosols of capsaicin (0.3 mM) at an airflow of 4 l min^{-1} to elicit coughing. The aerosol was generated by a jet nebulizer and the volume of solution aerosolized was approximately 0.4 ml min^{-1}. This dose of capsaicin will reliably elicit cough under these conditions (Bolser *et al.*, 1991a). Coughs were detected by a microphone placed in the chamber and connected to a audio monitor and chart recorder. The number of coughs elicited during a 4 min exposure to capsaicin were counted by visual inspection of the chart record.

The antitussive effect of codeine, dextromethorphan, GABA, baclofen, and 3-aminopropylphosphinic acid (3-

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APPi) was assessed following subcutaneous or aerosol administration of each drug. For the subcutaneous route of administration, animals were dosed 1 h before challenge with capsaicin. For aerosol administration, drugs or vehicle were delivered by inhalation for 4 min immediately before exposure to capsaicin aerosol. This method has been used in this laboratory to demonstrate blockade of capsaicin-induced cough *in vivo* by the capsaicin antagonist, ruthenium red (Bolser *et al.*, 1991a).

The activity of the $GABA_B$ antagonist, 3-aminopropyl (diethoxymethyl) phosphinic acid (CGP 35348), and a $GABA_A$ antagonist, bicuculline, were evaluated by the ability of these compounds to inhibit the antitussive effect of baclofen. These antagonists or vehicle were administered subcutaneously 40 min before challenge with capsaicin. Baclofen was administered subcutaneously 30 min before capsaicin challenge.

Mechanically induced cough in cats

Cats (2.2–4.0 kg) were anaesthetized with sodium pentobarbitone (35 mg kg^{-1}, i.p.). Supplemental anaesthetic (5 mg kg^{-1}, i.v.) was administered as required. Catheters were placed in a femoral vein and artery for administration of drugs and measurement of arterial blood pressure, respectively. A tracheal cannula was placed to allow access to the intrathoracic trachea.

Electromyograms (EMGs) of respiratory muscle activity were recorded via bipolar silver wire electrodes placed in the diaphragm and rectus abdominis muscles. The EMGs were amplified, filtered (0.5–10 kHz), monitored on a oscilloscope, and integrated with a resistance-capacitance circuit (100 ms time constant). These signals were displayed along the blood pressure on a chart recorder.

Cough is produced by coordinated bursts of activity in inspiratory and expiratory muscles (Korpas & Tomori, 1979; Tomori & Widdicombe, 1969). We defined cough as a burst of EMG activity in the diaphragm (inspiratory muscle) immediately followed by or coincident with burst of activity in the rectus abdominis muscle (expiratory muscle). These criteria are consistent with EMG recordings of respiratory muscle activity during coughing reported by other investigators (Tomori & Widdicombe, 1969; Korpas & Tomori, 1979; Van Lunteren *et al.*, 1989). These criteria also will differentiate coughs from apnoeas or apneusis (no expiratory bursts), augmented breaths (no expiratory bursts), or the expiration reflex (no inspiratory burst).

The antitussive activity of codeine, dextromethorphan, or baclofen was evaluated from cumulative dose-response relationships following intravenous administration of each drug. Coughing was elicited by probing the intrathoracic trachea with a thin flexible polyethylene cannula. Each cough trial consisted of continuous probing of the intrathoracic trachea for approximately 10 s. This stimulus only elicits coughs, augmented breaths (sighs), or the expiration reflex (Korpas & Tomori, 1979). Control values were obtained by averaging the number of coughs during five consecutive trials obtained following vehicle administration. One minute was allowed to elapse between trials. A total of five stimulus trials were then applied at 1,2,3,4, and 5 min after each dose of drug. The cough response following each dose of drug was determined by averaging the number of coughs observed during these five trials. Five minutes elapsed between each dose of drug.

$GABA_B$ antagonist activity was evaluated by administration of the $GABA_B$ antagonist CGP 35348 or the mixed agonist/antagonist (Hills & Howson, 1992) 3-aminopropylphosphonic acid (3-APPA) 5 min before baclofen. The antagonists and baclofen were given by the intravenous route. The number of coughs following mechanical stimulation was assessed as described above.

Compounds

Compounds used in this study included capsaicin, bicuculline methiodide (Sigma Chemical Co., St. Louis, MO, U.S.A.), CGP 35348 (Ciba-Geigy Corp., Basel, Switzerland), 3-APPA, dextromethorphan, naloxone HCl, and (±)-baclofen HCl (Research Biochemicals Inc., Natick, MA, U.S.A.), 3-APPi (Schering-Plough Research Institute, Bloomfield, NJ. U.S.A.) and codeine sulphate (Mallinckrodt, St. Louis, MO, U.S.A.). Capsaicin was dissolved in 1% ethanol, 1% Tween 20, and 98% physiological saline. All other drugs were dissolved in 0.9% saline.

Statistics

All data are represented as mean ± s.e.mean. Statistical differences between means were evaluated with Student's *t* test or one-way Analysis of Variance. Effective doses (ED_{50}, ED_{30}) were determined by linear regression analysis of dose-response relationships. Differences were considered significant if $P<0.05$.

Results

Irritant-induced cough in guinea-pigs

Figure 1 shows the antitussive effects of subcutaneous and aerosol administration of codeine, dextromethorphan and the $GABA_B$ agonists baclofen and 3-aminopropylphosphinic acid (3-APPi) against capsaicin-induced cough in the guinea-pig. The maximum inhibition of cough by these compounds in guinea-pigs by either route was 60–70%. Therefore, effective doses are expressed as ED_{30}'s in Table 1. Dose-dependent

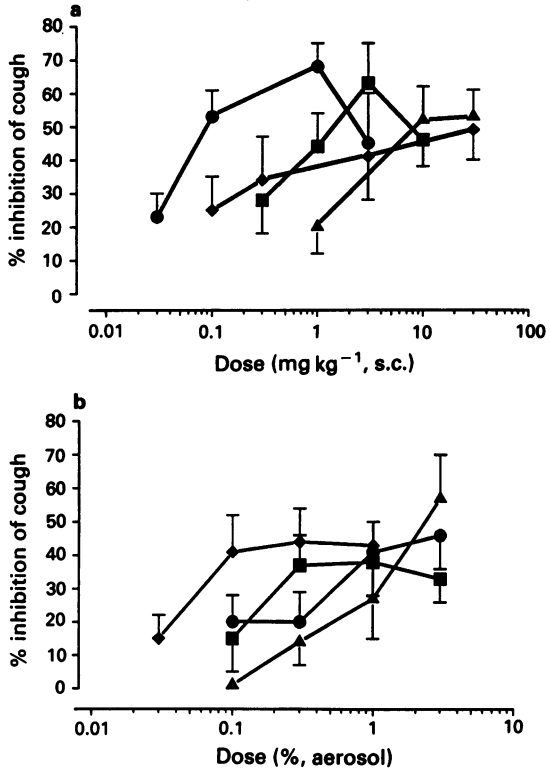

Figure 1 Influence of codeine, dextromethorphan and $GABA_B$ agonists administered via subcutaneous (a) and inhaled (b) routes on capsaicin-induced cough in the guinea-pig. Drugs were administered 1 h before capsaicin challenge in (a). In (b) drugs were delivered by aerosol for 4 min just before capsaicin challenge. Values represent mean ± s.e.mean ($n = 6–18$ animals per dose): (●) baclofen; (■) 3-aminopropylphosphinic acid; (▲) codeine; (◆) dextromethorphan.

Table 1 Antitussive potencies of $GABA_B$ agonists and selected standards in guinea-pigs

Compound[a]	*Inhibition of capsaicin-induced cough*[b] (ED_{30}) subcutaneous (mg kg^{-1})	inhaled (%)
Baclofen	0.04	0.59
3-APPi	0.36	0.22
Codeine	2.20	0.76
Dextromethorphan	0.31	0.08

[a]Compounds administered either 1 h before (subcutaneous) or 4 min before (inhaled) capsaicin challenge. [b]ED_{30} determined by regression analysis. $n = 24-72$ animals for each ED_{30} determination. 3-APPi = 3 aminopropylphosphinic acid.

inhibition was produced by each of these drugs by the subcutaneous route of administration (Figure 1a). Baclofen was apparently more potent than the other drugs by this route of administration (Table 1).

By the inhaled route, baclofen (0.1–3.0%) and 3-APPi (0.1–3.0%) each inhibited capsaicin-induced cough. All drugs had similar potencies to inhibit cough by the inhaled route (Figure 1b, Table 1). In addition, all drugs were equieffective in reducing cough by this route of administration.

The $GABA_B$ antagonist, CGP 35348 (0.3–30 mg kg^{-1}, s.c.) reduced the antitussive effect of baclofen (3 mg kg^{-1}, s.c., Figure 2) but did not alter the antitussive effect of codeine (30 mg kg^{-1}, s.c., Table 2). Conversely, naloxone at a dose (0.3 mg kg^{-1}, s.c.) that significantly inhibited the antitussive effect of codeine (30 mg kg^{-1}, s.c.) had no effect on the antitussive action of baclofen (Table 2). CGP 35348 alone had no effect on cough when delivered at doses up to 15 mg kg^{-1}, s.c. Pretreatment with the $GABA_A$ antagonist, bicuculline, (3 mg kg^{-1}, s.c.) had no effect on the antitussive activity of baclofen (baclofen alone 41 ± 18% inhibition of cough, baclofen + bicuculline 43 ± 13% inhibition).

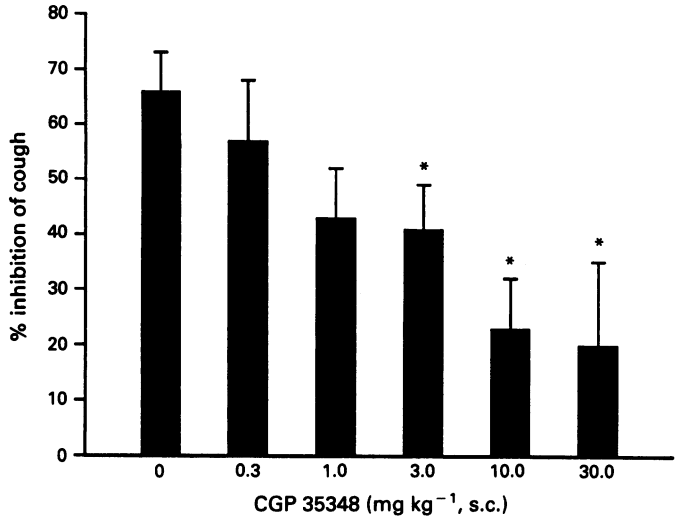

Figure 2 Influence of the $GABA_B$ antagonist CGP 35348 on the antitussive effect of baclofen in the guinea-pig. CGP 35348 was administered subcutaneously 40 min before challenge with capsaicin aerosol. Baclofen (3 mg kg^{-1}) was administered subcutaneously 30 min before challenge with capsaicin aerosol. The inhibition of cough frequency produced by baclofen in the absence of CGP 35348 was 66 ± 7%. Values represent mean ± s.e.mean ($n = 6-18$ per group). *$P < 0.05$ compared to baclofen alone.

Table 2 Effects of selective antagonists on the antitussive effects of baclofen and codeine in guinea-pigs

Treatment *First*[a]	*Second*[b]	*% inhibition of cough (mean ± s.e.mean)*	
CGP35348	Saline	9 ± 21[c]	
Saline	Codeine	44 ± 14	NS
CGP35348	Codeine	61 ± 21	
Naloxone	Saline	27 ± 8[c]	
Saline	Codeine	62 ± 13	*
Naloxone	Codeine	17 ± 6	
Naloxone	Saline	−23 ± 29[c]	
Saline	Baclofen	58 ± 12	NS
Naloxone	Baclofen	68 ± 6	

[a]Naloxone (0.3 mg kg^{-1}, s.c.), CGP35348 (10 mg kg^{-1}, s.c.), or saline was given 40 min before capsaicin challenge. [b]Codeine (30 mg kg^{-1}, s.c.), baclofen (3.0 mg kg^{-1}, s.c.), or saline was given 30 min before capsaicin challenge. [c]Naloxone or CGP35348 had no significant effects on capsaicin-induced cough.
NS = not significant $n = 5-6$ per group. * = $P < 0.05$

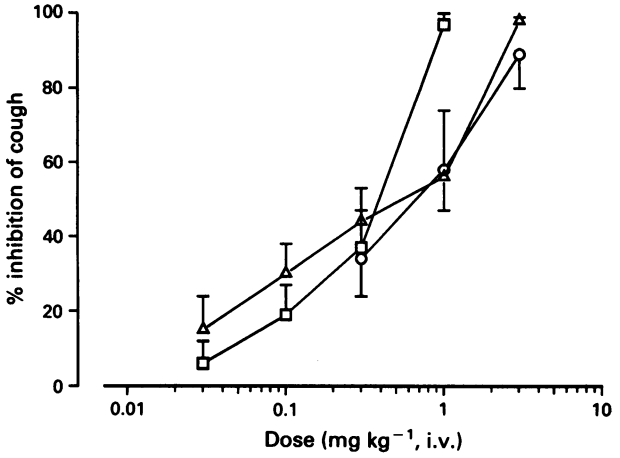

Figure 3 Cumulative dose-response relationships for codeine, dextromethorphan, and baclofen on mechanically-induced cough in the cat: (○) baclofen; (△) codeine; (□) dextromethorphan. Values represent mean ± s.e.mean ($n = 5$ animals per drug).

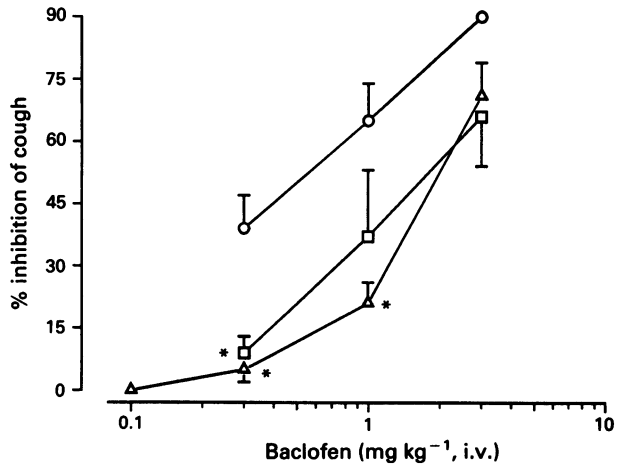

Figure 4 Influence of $GABA_B$ antagonists CGP 35348 and 3-aminopropylphosphonic acid (3-APPA) on antitussive effect of baclofen in the cat. Antagonists or vehicle administered 5 min before first dose of baclofen: (○) vehicle and baclofen; (△) CGP 35348 (10 mg kg^{-1}, i.v.) and baclofen; (□) 3-APPA (3 mg kg^{-1}, i.v.) and baclofen. Values represent mean ± s.e.mean ($n = 5$ animals per drug). *$P < 0.05$ compared to vehicle and baclofen.

Mechanically-induced cough in the cat

Intravenous administration of baclofen (0.3–3 mg kg^{-1}), codeine (0.03–3.0 mg kg^{-1}), or dextromethorphan (0.03–1.0 mg kg^{-1}) reduced mechanically-induced cough in a dose-dependent manner in the cat (Figure 3). The maximum inhibition produced by these drugs was 90–100% and half-maximum inhibition (ED_{50}) was produced by 0.63 mg kg^{-1} baclofen, 0.26 mg kg^{-1} codeine, and 0.27 mg kg^{-1} dextromethorphan.

Prior administration of the $GABA_B$ antagonists, 3-APPA (3 mg kg^{-1}, i.v.) or CGP 35348 (10 mg kg^{-1}, i.v.), shifted the dose-response relationship for baclofen to the right (Figure 4). A higher dose of 3-APPA (10 mg kg^{-1}, i.v.) inhibited the cough response to mechanical stimulation of the trachea, suggestive of agonist activity by this mixed agonist/antagonist.

Discussion

In our studies, baclofen and 3-APPi, which are highly specific $GABA_B$ receptor agonists (Bowery, 1989; Hills & Howson, 1992), inhibited cough in cats and guinea-pigs and the antitussive effect of baclofen was antagonized by selective $GABA_B$ antagonists. In contrast, the $GABA_A$ antagonist, bicuculline, and the opioid antagonist, naloxone, did not influence the antitussive effect of baclofen in the guinea-pig. Likewise, the $GABA_B$ antagonist, CGP 35348, did not alter the antitussive activity of codeine. These observations indicate that the antitussive effects of $GABA_B$ agonists are specific to $GABA_B$ receptors and are independent of $GABA_A$ or opioid receptors. Previously, Nosalova *et al.* (1987) showed that a nonspecific GABA agonist, gabalineamide, had antitussive effects in the cat. But it was not known if the antitussive effect observed by Nosalova *et al.* was due to $GABA_A$ or $GABA_B$ receptors. Our results clearly define a role for inhibition of cough by $GABA_B$ receptor agonists in cats.

Baclofen and 3-APPi had antitussive activity when given systemically or by inhalation to guinea-pigs. This observation contrasts with the findings of Callaway & King (1992) who showed that inhaled baclofen and GABA did not inhibit citric acid-induced cough in guinea-pigs. These differences in results could be due to different protocols and/or the different stimuli (citric acid, capsaicin) used to elicit cough. In our studies, we compared the effects of selective $GABA_B$ agonists to the well-characterized drugs, codeine and dextromethorphan. The antitussive effects of codeine and dextromethorphan that we demonstrated in the guinea-pig are generally consistent with a variety of reports showing similar effects of these drugs on cough induced by inhaled irritants in rats and guinea-pigs (Pickering & James, 1979; Adcock *et al.*, 1988; Karlsson *et al.*, 1990; Kamei & Kasuya, 1992). However caution should be used in comparing antitussive effects of drugs on cough elicited by different irritant stimuli, such as citric acid and capsaicin. Although previous investigators have suggested that citric acid and capsaicin act by the same mechanism on the basis of neuropeptide depletion experiments using capsaicin (Forsberg & Karlsson, 1986), we have recently shown that citric acid can elicit cough by the same or different mechanisms as capsaicin depending upon the dose of citric acid administered (Bolser *et al.*, 1991a). These observations underscore the importance of using the same tussigenic stimulus when comparing activities of antitussive drugs.

The potencies of baclofen and 3-APPi when administered by the subcutaneous or inhaled routes in the guinea-pig compared favourably to that of the standard antitussives, codeine and dextromethorphan. The maximum efficacy of baclofen and 3-APPi in the guinea pig model was approximately 60% inhibition of cough. This observation suggests that it may be difficult to inhibit completely capsaicin-induced cough in this species. However, baclofen and 3-APPi had efficacies equivalent to or greater than codeine and dextromethorphan in this model.

In the cat, the antitussive potencies of codeine and dextromethorphan in the present study are similar to or greater than potencies reported by others (May & Widdicombe, 1954; Chau & Harris, 1980; Kase *et al.*, 1983). In addition, in a model of fictive cough (Bolser, 1991) we have previously reported a potency of codeine (0.1 mg kg^{-1}, i.v.) similar to that found in the present study. Furthermore, dextromethorphan was more potent in our studies (ED_{50} = 0.27 mg kg^{-1}, i.v.) than was reported previously (ED_{50} = 0.82–1.21 mg kg^{-1}; Chau & Harris, 1980; Domino *et al.*, 1985). Therefore, our cat model appears to be very sensitive to the effects of antitussive drugs. In this model, the potency of baclofen was only slightly less than codeine and dextromethorphan.

Previous investigators have suggested peripheral and central sites of action for antitussive drugs (Chou & Wang, 1975; Karlsson *et al.*, 1990). The fact that 3-APPi inhibits cough is consistent with a peripheral site of action, because 3-APPi does not penetrate the CNS (Hills & Howson, 1992). Indeed, we have also found that large doses (30–100 mg kg^{-1}, s.c.) of 3-APPi do not cause respiratory depression in guinea-pigs whereas baclofen (3.0 mg kg^{-1}, s.c.) causes significant respiratory depression (J.A. Hey, G. Mingo & R.W. Chapman; unpublished observations). Therefore, a peripheral site of action of 3-APPi to inhibit cough seems likely. Whether or not baclofen also acts at central sites to inhibit cough is unknown.

The antitussive effects of $GABA_B$ agonists are consistent with their inhibitory effects on other phenomena that are important in the pathogenesis of airway diseases. Baclofen decreases cholinergic and tachykinin-mediated bronchospasm (Belvisi *et al.*, 1989; Chapman *et al.*, 1991) and attenuates airway microvascular leakage induced by stimulation of tachykinin-containing sensory afferents (Danko *et al.*, 1992). Furthermore, baclofen inhibits allergen and histamine-induced bronchospasm in conscious guinea-pigs and attenuates the release of allergic mediators from guinea-pig lungs (Luzzi *et al.*, 1987). Therefore, $GABA_B$ agonists appear to be active in reducing a variety of components that contribute to the pathogenesis of airway diseases, such as asthma.

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