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Peripheral and central sites of action of GABA-B agonists to inhibit the cough reflex in the cat and guinea pig

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1 The GABA-B receptor agonists baclofen and 3-aminopropylphosphinic acid (3-APPi) have antitussive activity in the cat and guinea pig. The purpose of this study was to investigate the sites of action of these GABA-B receptor agonists to inhibit the cough reflex.

2 Single intracerebroventricular (i.c.v.) cannulas were placed in the lateral ventricles of anaesthetized guinea pigs. Approximately 1 week later, the animals were exposed to aerosols of capsaicin (0.3 mM) to elicit coughing. Coughs were detected with a microphone and counted.

3 Cough was produced in anaesthetized cats by mechanical stimulation of the intrathoracic trachea and was recorded from electromyograms of respiratory muscle activity. Cannulas were placed for intravenous (i.v.) or, in separate groups of animals, intravertebral arterial (i.a.) administration of baclofen, 3-APPi, the centrally active antitussive drug codeine or the peripherally active antitussive drug BW443c. Dose–response relationships for i.v. and i.a. administration of each drug were generated to determine a ratio of i.v. ED_{50} to i.a. ED_{50}, known as the effective dose ratio (EDR). The EDR will be 20 or greater for a centrally acting drug.

4 In the guinea pig, baclofen (3 mg kg^{-1}, s.c.) and 3-APPi (10 mg kg^{-1}, s.c.) inhibited capsaicin-induced cough by 50% and 35% respectively. The antitussive activity of baclofen was completely blocked by i.c.v. administration of the GABA-B receptor antagonist CGP 35348 (10 μg). Conversely, the antitussive effect of 3-APPi was unaffected by i.c.v. CGP 35348. However, systemic administration of CGP 35348 (30 mg kg^{-1}, s.c.) completely blocked the antitussive activity of 3-APPi (10 mg kg^{-1}, s.c.). In separate experiments baclofen alone (1 μg, i.c.v.) inhibited capsaicin-induced cough by 78%. 3-APPi (10 and 100 μg, i.c.v.) had no effect on capsaicin-induced cough in the guinea pig.

5 In the cat, potencies (ED_{50}) of the standards and GABA-B agonists by the i.v. route were: codeine (0.34 mg kg^{-1}), BW443C (0.17 mg kg^{-1}), baclofen (0.63 mg kg^{-1}) and 3-APPi (2.3 mg kg^{-1}). Potencies of these drugs by the i.a. route were: codeine, 0.013 mg kg^{-1}; BW443C, 0.06 mg kg^{-1}; baclofen, 0.016 mg kg^{-1}; and 3-APPi, 0.87 mg kg^{-1}. The EDRs for each drug were: codeine, 26; BW443C, 3; baclofen, 39; and 3-APPi, 3.

6 We conclude that in both the cat and guinea pig baclofen inhibits cough by a central site of action, while 3-APPi inhibits cough by a peripheral site of action.

Keywords: Cough; antitussive; GABA-B receptors; baclofen; 3-aminopropylphosphinic acid

Introduction

The selective GABA-B receptor agonists baclofen and 3-aminopropylphosphinic acid, (3-APPi) have recently been shown to have antitussive activity in the cat and guinea pig (Bolser *et al.*, 1993a). These effects are specific to activation of GABA-B receptors because the antitussive effects of baclofen were blocked by the GABA-B receptor antagonists CGP 35348 and 3-aminopropylphosphonic acid (Bolser *et al.*, 1993a). Possible sites of action of antitussive drugs include the inhibition of one or more components of the central reflex pathway for cough located primarily in the medulla (Chou & Wang, 1975; Korpas & Tomori, 1979; Adcock, 1991) or inhibition of the responsiveness of sensory receptors involved in the generation of cough (Adcock, 1991; Korpas & Tomori, 1979). For example, the well-known antitussive drug codeine inhibits cough at central sites (Chou & Wang, 1975), whereas other antitussive drugs, such as the opioid μ-receptor agonist BW443C, have only a peripheral site of action (Adcock *et al.*, 1988).

In this study, we investigated the sites of action of baclofen and 3-APPi in inhibiting the cough reflex. For GABA-B agonists, it is likely that baclofen acts centrally to inhibit the cough reflex, because this drug readily penetrates the CNS and can alter the discharge of spinal respiratory neurones (Lalley, 1983). Conversely, 3-APPi would be expected to have a peripheral site of action in inhibiting cough, because this drug does not penetrate the CNS (Hills & Howson, 1992). We established the central or peripheral site of action of these agents in guinea pigs by evaluating the ability of intracerebroventricular administration of the GABA-B antagonist CGP 35348 to block the antitussive effects of baclofen or 3-APPi. Furthermore, antitussive potencies were compared in cats after intravenous or intra-arterial injection of GABA-B agonists. A preliminary account of this work has been published (Bolser *et al.*, 1993b).

Methods

Capsaicin-induced cough in the guinea pig

Male unanaesthetized Dunkin–Hartley guinea pigs (500–800 g) were placed in a cylindrical transparent chamber and individually exposed to capsaicin aerosols (0.3 mM) to elict cough (Bolser *et al.*, 1993a). The dimensions of the chamber were 12" × 4". The capsaicin aerosol was produced by a jet nebulizer at an airflow of 4 l min^{-1}. The volume of capsaicin nebulized was approximately 1.6 ml. A microphone was placed in the chamber to detect coughs, and this microphone was connected to a chart recorder. The animals were exposed

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to capsaicin for 4 min and the number of coughs elicited during this period was counted by visual inspection of the chart record.

Intracerebroventricular (i.c.v) administration of the GABA-B receptor antagonist, 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP 35348), was used to evaluate the central antitussive action of systemically administered baclofen and 3-APPi. Single i.c.v. cannulae were placed into the lateral ventricle of anaesthetized (ketamine, 30 mg kg^{-1}; xylazine, 5 mg kg^{-1} i.m.) guinea pigs and anchored in place with dental cement. Coordinates for cannula placement were: 0.5 mm anterior (A), 2.0 mm lateral (L), and 4.5 mm ventral (V) relative to bregma (McLeod *et al.*, 1991). The animals were allowed to recover and approximately 1 week later were used for cough experiments. Baclofen (3.0 mg kg^{-1} s.c.) or 3-APPi (10 mg kg^{-1} s.c.) was administered 30 min before exposure to capsaicin. These doses were chosen on a previous study that demonstrated antitussive activity of baclofen and 3-APPi in guinea pigs (Bolser *et al.*, 1993a). CGP 35348 (10 μg in 10 μl) or artificial CSF (10 μl) was given i.c.v. 10 min before exposure to capsaicin. This dose of CGP 35348 was selected because it is much lower than the minimally active systemic dose of 3 mg kg^{-1} (Bolser *et al.*, 1993a) and preliminary experiments showed that it does not alter the cough reflex. In separate deperiments, baclofen (1 μg in 10 μl) and 3-APPi (10 and 100 μg in 10 μl) were given i.c.v. 5 min before exposure to capsaicin.

Additional experiments were conducted to verify that the antitussive effects of 3-APPi could be antagonized by systemic administration of CGP 35348. CGP 35348 (30 mg kg^{-1} s.c.) was administered 40 min before capsaicin challenge. 3-APPi (10 mg kg^{-1} s.c.) was then administered 30 min before capsaicin challenge.

Mechanically induced cough in cats

Cats (2.5–5.0 kg) were anaesthetized with pentobarbital sodium (35 mg kg^{-1} i.p.) and given supplemental anaesthetic as necessary (5 mg kg^{-1} i.v.). Atropine sulphate (1.0 mg kg^{-1} i.v.) was administered to block reflex tracheal secretions. The trachea, femoral vein and femoral artery were cannulated. In some animals, a catheter was inserted into the left axillary artery and advanced until the catheter tip was at the branch of the left vertebral artery (Chou & Wang, 1975). The omocervical, pericardiophrenic and costocervical branches were ligated. Evans blue dye was injected into the catheter at the end of the experiment and proper placement of this catheter was confirmed post mortem.

Bipolar silver wire electrodes were placed in the diaphragm and rectus abdominis muscles by the technique of Basmajian & Stecko (1962). Electromyograms (EMGs) from these muscles were amplified, filtered (0.5–10 kHz), and integrated with a resistance–capacitance circuit (100 ms time constant). The integrated EMGs were displayed on a chart recorder.

Cough was defined as a burst of EMG activity in the diaphragm immediately followed by a burst of EMG activity in the rectus abdominis muscle (Bolser *et al.*, 1993a). Coughing was produced by probing the intrathoracic trachea with a thin flexible polyethylene cannula continuously for approximately 10 s per trial. Control values were generated by averaging the number of coughs during five consecutive probing trials obtained after vehicle administration. One minute elapsed between probing trials. Probing trials were applied at 1-min intervals after each dose of drug for a total of five stimulus trials between doses. The cough response after each dose of drug was determined by averaging the number of coughs observed during these five probing trials. Five minutes elapsed between each dose of drug.

The antitussive activities of codeine, the peripheral μ-opioid agonist BW443C, baclofen and 3-APPi were evaluated from cumulative dose–response relationships after intravenous (i.v.) administration and, in separate groups of animals, after intra-arterial (i.a.) administration of each drug. An effective dose ratio (EDR) was generated for each drug. The EDR was defined as the ED_{50} for i.v. activity divided by the ED_{50} for i.a. activity of the drug. This ratio will be 20 or greater for a centrally acting antitussive drug and less than 20 for a peripheral antitussive drug (Chou & Wang, 1975).

Compounds

Compounds used in this study included atropine sulphate and capsaicin (Sigma Chemical Co., St Louis, MO, U.S.A.), codeine sulphate (Mallinkrodt, St Louis, MO, U.S.A.), BW443C (L-tyrosyl-D-arginylglycyl-4-nitrophenylalanyl-L-proliamide diacetate) (a kind gift from Wellcome Research Laboratories, Beckenham, Kent, U.K.), (±) baclofen (Research Biochemicals, Natick, MA, U.S.A.), CGP 35348 and 3-APPi (prepared at Schering-Plough Research Institute, Kenilworth, NJ, U.S.A.). Capsaicin was dissolved in 1% ethanol, 1% Tween 20 and 0.9% physiological saline. All other drugs were dissolved in physiological saline. Doses were calculated as their free base.

Statistics

All data are expressed as mean ± s.e. mean. Student's *t*-test or one-way analysis of variance was used to evaluate differences between means. Effective doses (ED_{50}) for 50% inhibition were obtained by regression analysis of dose–response relationships. Differences between means were considered significant if $P<0.05$.

Results

Influence of i.c.v. CGP 35348 on the antitussive effects of baclofen and 3-APPi in the guinea pig

Baclofen (3.0 mg kg^{-1} s.c.) inhibited capsaicin-induced cough by approximately 50% in the animals treated with i.c.v. artificial CSF (Figure 1). This antitussive effect of baclofen was completely blocked by i.c.v. administration of 10 μg of CGP 35348 (Figure 1).

3-APPi (10 mg kg^{-1} s.c.) inhibited capsaicin-induced cough by approximately 35% in the animals treated with i.c.v. artificial CSF (Figure 2). However, i.c.v. administration of CGP 35348 (10 μg) did not block the antitussive activity of 3-APPi (Figure 2). Intracerebroventricular administration of CGP 35348 alone had no significant effect on the cough reflex (i.c.v. artificial CSF 12 ± 1 coughs, $n = 22$; i.c.v. CGP 35348 11 ± 1 coughs, $n = 18$).

Influence of systemic CGP 35348 on the antitussive effect of 3-APPi in the guinea pig

3-APPi (10 mg kg^{-1} s.c.) inhibited capsaicin-induced cough by 57% (Table 1). Pretreatment with CGP 35348 (30 mg kg^{-1} s.c.) completely blocked the antitussive effects of 3-APPi (Table 1).

Influence of i.c.v. baclofen and 3-APPi on capsaicin-induced cough in the guinea pig

Baclofen (1 μg i.c.v.) inhibited capsaicin-induced cough by approximately 70% (Table 2). However, i.c.v. administration of 3-APPi at doses of 10 and 100 μg had no antitussive effect.

Intravenous and intra-arterial administration of baclofen, 3-APPi, codeine and BW443C in the cat

In animals that received drugs i.v., mechanical stimuli applied to the trachea elicited 7.3 ± 0.3 coughs per stimulus trial after saline administration ($n = 22$). In animals that

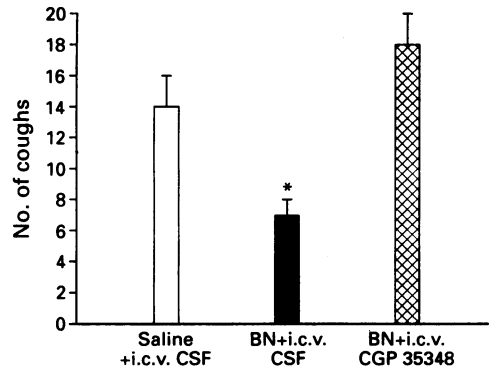

Figure 1 Influence of i.c.v. CGP 35348 on the antitussive activity of systemic baclofen in the guinea pig. Saline or baclofen (3.0 mg kg^{-1} s.c.) were given 30 min before capsaicin challenge. Artificial CSF (10 μg in 10 μl i.c.v.) or CGP 35348 (10 μl i.c.v.) was given 10 min before capsaicin challenge. *$P<0.05$, $n=4-11$ per group.

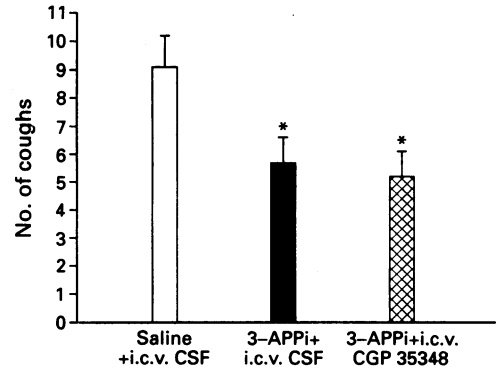

Figure 2 Influence of i.c.v. CGP 35348 on the antitussive activity of systemic 3-APPi in the guinea pig. Saline or 3-APPi (10 mg kg^{-1} s.c.) was given 30 min before capsaicin challenge. Artificial CSF (10 μl i.c.v.) or CGP 35348 (10 μg in 10 μl i.c.v.) was given 10 min before capsaicin challenge. *$P<0.05$, $n=9-13$ per group.

received drugs i.a., mechanical stimuli applied to the trachea elicited 8.8 ± 0.4 coughs per stimulus trial after saline administration ($n=23$). Figure 3 shows cumulative dose–response relationships for antitussive activity after i.v. and i.a. administration of codeine (Figure 3a), BW443C (Figure 3b), baclofen (Figure 3c) and 3-APPi (Figure 3d). Each dose–response relationship was generated from a separate group of animals.

Codeine was much more potent when given by the i.a. route ($ED_{50}=0.013$ mg kg^{-1}) than by the i.v. route ($ED_{50}=0.34$ mg kg^{-1}). The EDR for codeine was 26. The peripherally acting opioid BW443C was slightly more potent when given by the i.a. route ($ED_{50}=0.06$ mg kg^{-1}) than by the i.v. route ($ED_{50}=0.17$ mg kg^{-1}1) and had an EDR of 3.0. Baclofen was much more potent when given by the i.a. route ($ED_{50}=0.016$ mg kg^{-1}) than by the i.v. route ($ED_{50}=0.63$ mg kg^{-1}). The EDR for baclofen was 39. Conversely, 3-APPi was only slightly more potent by the i.a. ($ED_{50}=0.87$ mg kg^{-1}) route than by the i.v. route ($ED_{50}=2.3$ mg kg^{-1}) and had an EDR of 3.0. 3-APPi also was less efficacious than the other drugs, producing a maximum inibition of cough frequency of approximately 60%.

Discussion

The major findings of this study were that central administration of a GABA-B antagonist, CGP 35348, reversed the antitussive effect of systemically administered baclofen, but not that of 3-APPi in the guinea pig. Furthermore, i.c.v. administration of baclofen, but not 3-APPi, inhibited cough in the guinea pig. In the cat, the EDR for i.v. and i.a. administration of baclofen was similar to that of the central antitussive drug, codeine. However, the EDR for i.v. and i.a. 3-APPi was similar to that of BW443C, a peripheral antitussive drug.

Central administration of antagonists is a standard approach to evaluate the site of action of systemically administered drugs. The antagonist must be administered at doses that are inactive when given systemically. In this study, CGP 35348 (10 μg i.c.v.) completely blocked the antitussive activity of baclofen. This dose of CGP 35348 was at least 300-fold smaller than the effective systemic dose to block baclofen (Bolser *et al.*, 1993a). This indicates that baclofen inhibited capsaicin-induced cough in the guinea pig by a central site of action and that there was no peripheral component to the action of this GABA-B agonist. Conversely, while i.c.v. CGP 35348 did not reverse the antitussive action of 3-APPi, systemic administration of this antagonist did block the antitussive effects of 3-APPi. There are two alternative interpretations of these observations. First, 3-APPi had solely a peripheral site of action so its antitussive activity was unaffected by central administration of CGP 35348. Second, in addition to a peripheral action 3-APPi acted at a central GABA-B receptor subtype that was insensitive to CGP 35348. To resolve these two alternative interpretations, we administered baclofen and 3-APPi i.c.v. in the guinea pig. While baclofen (1 μg) inhibited cough when given i.c.v., 3-APPi was inactive at doses up to 100 μg. These data indicate that 3-APPi did not have a central action to inhibit cough in the guinea pig. However, the failure of i.c.v. CGP 35348 to block the antitussive effects of 3-APPi cannot be attributed to a lack of central penetration by this GABA-B agonist. Even if a systemic dose of 3-APPi penetrated the CNS in our

Table 1 Influence of systemic CGP 35348 on the antitussive effect of 3-APPi

Treatment[a]			
First	*Second*	n	*No. of coughs (mean ± s.e.mean)*
Saline	Saline	10	14 ± 1
Saline	3-APPi	10	6 ± 2*
CGP 35348	3-APPi	10	15 ± 2

[a]The first treatment was administered 40 min before capsaicin challenge. The second treatment was administered 30 min before capsaicin challenge. All treatments were administered by the subcutaneous route. The dose of 3-APPi was 10 mg kg^{-1} and the dose of CGP 35348 was 30 mg kg^{-1}.
*$P<0.05$ relative to either the saline/saline or the CGP 35348/3-APPi group.

Table 2 Influence of intracerebroventricular 3-APPi and baclofen on capsaicin-induced cough in the guinea pig

Treatment[a]	Dose (μg)	n	*No. of coughs (mean ± s.e.mean)*	
CSF	–	6	13 ± 2	*
Baclofen	1	5	3 ± 1	
CSF	–	9	10 ± 2	NS
3-APPi	10	9	9 ± 1	
CSF	–	6	8 ± 1	NS
3-APPi	100	5	8 ± 1	

[a]Compounds administered i.c.v. in 10 μl of artificial CSF 5 min before capsaicin challenge.
NS, not significant. *$P<0.05$.

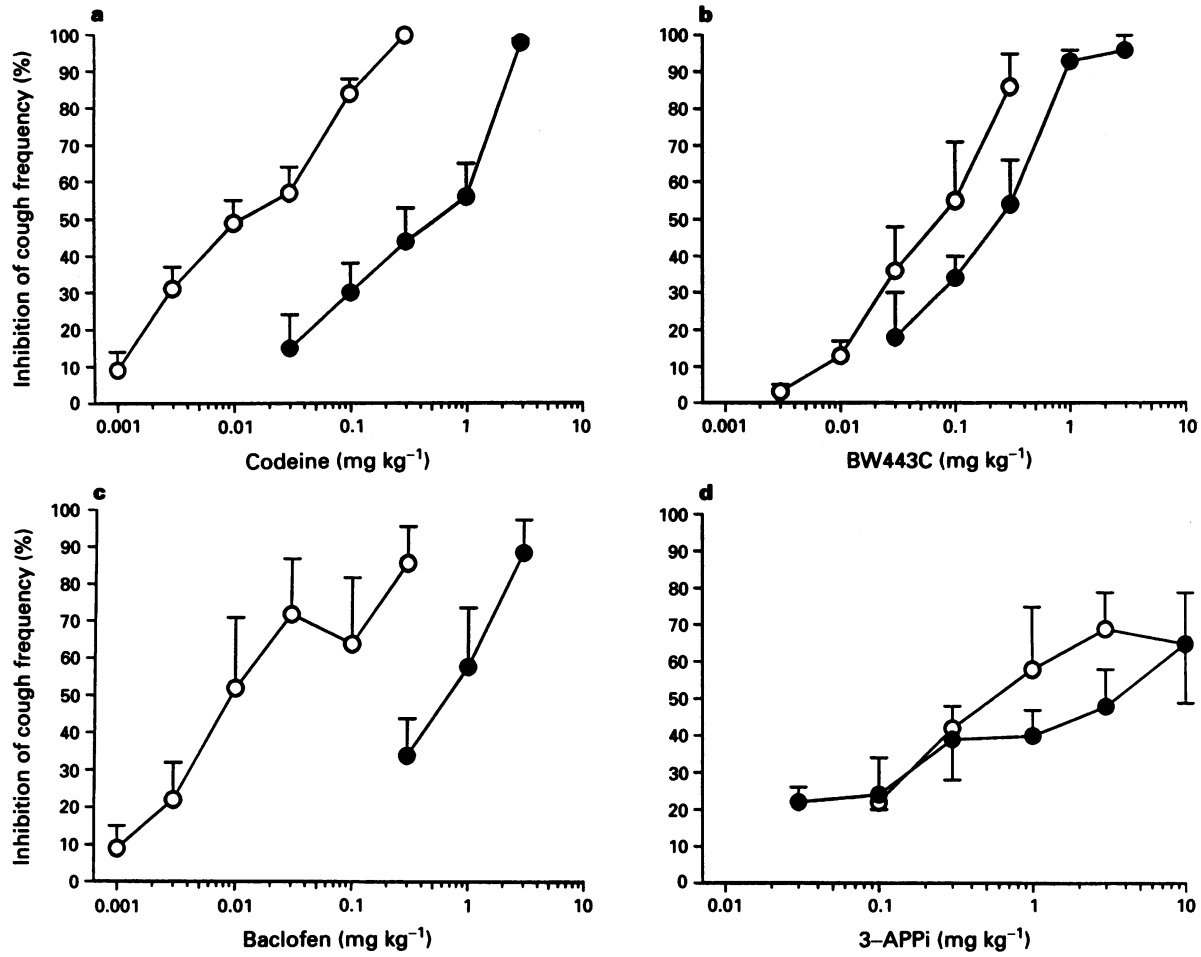

Figure 3 Effect of i.v. or i.a. GABA-B agonists and selected standards on cough frequency in the cat. Cumulative dose–response relationships for i.v. (●) and i.a. (○) administration of codeine (a), BW 443C (b), baclofen (c) and 3-APPi (d) are shown.

model, it probably would not have an antitussive effect. These results are consistent with baclofen preferentially acting at a central site that is unrecognized by 3-APPi. Our results indicate that, when 3-APPi and baclofen are given systemically, 3-APPi inhibits cough at a peripheral site of action that is relatively insensitive to baclofen. Conversely, baclofen inhibits cough at a central site that is relatively insensitive to 3-APPi.

The use of effective dose ratios generated from i.v. and i.a. (vertebral artery) dose–response relationships has been demonstrated to differentiate between centrally and peripherally active antitussive drugs (Chou & Wang, 1975). The EDR for centrally active antitussive drugs is generally high (greater than 20) because very small doses of these drugs are necessary to inhibit cough when injected into the vertebral artery. This artery supplies the brain stem, which is the primary site where the central neural elements generating cough are located (Chou & Wang, 1975). In contrast, much larger doses of peripheral antitussive drugs are necessary to inhibit cough when given in the vertebral artery because these drugs must recirculate in the venous system to reach their site of action. EDRs for peripheral antitussive drugs are less than 20 (Chou & Wang, 1975). In these studies, the EDR for baclofen was 39 and that of codeine was 26. This observation indicates that baclofen had a central site of action to inhibit the cough in the cat. The EDR for 3-APPi was only 3, the same as that of BW443C. These findings indicate that the antitussive activity of 3-APPi in the cat is also through a peripheral site of action.

Taken together, the results of this study indicate that in more than one species the antitussive activity of baclofen has a central component and that of 3-APPi is due to a peripheral site of action. These conclusions confirm and extend a previous suggestion that baclofen inhibits cough at a central site and that 3-APPi inhibits cough at a peripheral site (Chapman *et al.*, 1993). However, this suggestion was based primarily on the idea that 3-APPi penentrates the CNS to a limited extent (Hills & Howson, 1992). The data presented are the first direct evidence to support the conclusion that baclofen can inhibit cough by a central site of action and that 3-APPi inhibits cough solely by a peripheral site of action. The mechanism by which baclofen elicits its central antitussive effect is probably inhibition of one or more components of the central reflex pathway for cough. Conversely, 3-APPi probably elicits its peripheral antitussive effect by inhibition of sensory afferent responsiveness to inhaled capsaicin in the guinea pig or to mechanical stimulation of the trachea in the cat. In the guinea pig, inhaled capsaicin is thought to stimulate the C-fibers to elicit cough (Forsberg *et al.*, 1988). Pulmonary rapidly adapting receptors (RARs) have long been associated with the production of cough and are thought to be primarily responsible for cough elicited by mechanical stimuli applied to the intrapulmonary airway (Karlsson *et al.*, 1988). Based on this evidence, it is likely that 3-APPi can inhibit the responsiveness of more than one type of vagal afferent to mechanical and chemical stimuli. Similar mechanisms have been suggested for the peripheral antitussive drugs BW443C (Adcock *et al.*, 1988) and benzonatate (Korpas & Tomori, 1979).

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