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Effects of moguisteine on the cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve in guinea pigs

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Abstract

The study aimed to further demonstrate the peripheral antitussive properties of moguisteine. Firstly, the antitussive effect of moguisteine on the cough reflex induced by inhalation of citric acid aerosol was evaluated in conscious guinea pigs. Secondly, the effects of both moguisteine and codeine on the centrally mediated cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve were investigated in anesthetized guinea pigs. Moguisteine (2.5–10 mg/kg, intravenously, i.v.) reduced the cough reflex induced by 7.5% citric acid aerosol in a dose-dependent manner, with an ED_{50} value of 0.55 mg/kg. Both i.v. (0.5–4 mg/kg) and intracerebroventricular (i.c.v., 5–20 μg) injection of codeine dose dependently inhibited the cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve; the ED_{50} values were 0.91 mg/kg and 7.90 μg, respectively. The inhibitory effect of codeine (4 mg/kg i.v.) was abolished by pretreatment with naloxone (2 mg/kg intraperitoneally). In contrast to codeine, neither i.v. (4 and 20 mg/kg) nor i.c.v. (20 μg) injection of moguisteine affected the cough reflex. These results suggest that the antitussive effect of codeine is mediated by central opioid mechanisms, whereas the antitussive effect of moguisteine is mediated by peripheral mechanisms.

Keywords: Moguisteine; Cough; Antitussive drug; Codeine; Naloxone; Superior laryngeal nerve

1. Introduction

Moguisteine, (*R,S*)-2-(2-methoxyphenoxy)-methyl-3-ethoxycarbonyl-acetyl-1,3 thiazolidine, is a novel antitussive drug that is as active as codeine in several experimental models of cough induced by inhalation of chemical irritants such as citric acid and capsaicin, or by mechanical or electrical stimulation of the trachea (Gallico et al., 1994). In contrast to codeine, the antitussive effect of moguisteine is not inhibited by naloxone, an opioid receptor antagonist, and intracerebroventricular (i.c.v.) injection of the drug has no antitussive effect (Gallico et al., 1994). Moreover, recent reports have documented that moguisteine inhibits the activity of rapidly adapting receptors, which are one of the types of airway receptors associated with the cough reflex, in guinea pigs (Morikawa et al., 1997) and dogs (Sant'Ambrogio and Sant'Ambrogio, 1998). On the basis of these reports, it is thought that the antitussive effect of moguisteine is mediated by peripheral and non-opioid mechanisms.

Originating in the larynx, the afferent pathway that is mainly responsible for most of the reflex responses, including cough and bronchoconstriction, runs in the superior laryngeal nerve (Karlsson et al., 1988). Therefore, afferent electrical stimulation of the superior laryngeal nerve can evoke a cough reflex in cats (Domenjoz, 1952). This cough model is suitable not only for evaluating the efficacy of centrally acting antitussive drugs, but also for determining whether or not the site of action of antitussive drugs is central. In the present study, we firstly evaluated the antitussive effect of moguisteine administered intravenously (i.v.) on the cough reflex induced by inhalation of citric acid aerosol in conscious guinea pigs. Secondly, to further demonstrate the peripheral antitussive properties of moguisteine, we attempted to reproduce the model of cough induced by afferent electrical stimulation of the superior laryngeal nerve in anesthetized guinea pigs, and to

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investigate the effects of both i.v. and i.c.v. injections of moguisteine and codeine, as a positive reference drug, in this model.

2. Materials and methods

2.1. Animals

Female Dunkin–Hartley guinea pigs (Rodentia, Bergamo, Italy) weighing 300–400 g were used for experiment 1. The animals were maintained in conditioned quarters (temperature of 21 ± 2°C, relative humidity of 55 ± 10% and a 12-h light/dark cycle) with free access to food and water for at least 1 week before use. Male Std.: Hartley guinea pigs (Japan SLC, Hamamatsu, Japan) aged 4 or 5 weeks and weighing 250–350 g were used for experiment 2. They were housed 3 to 4 per cage under controlled ambient temperature (23 ± 2°C) and relative humidity (60 ± 10%) with a 12-h light/dark cycle (lights on 0700 h) for at least 1 week prior to use. Food and water were available ad libitum. The animals were used for experiment 2 when they were 5–8 weeks old.

2.2. Experiment 1: inhalation of citric acid aerosol

The method used was that described by Charlier et al. (1961) with minor modifications. One day before the experiment, the right jugular vein was cannulated for drug administration under pentobarbital sodium (25 mg/kg, intraperitoneally, i.p.) anesthesia. Twenty-four hours later, the animals were randomly assigned to the experimental groups (8 animals/dose) and treated with the drug 5 min before testing. Thereafter, each animal was placed in a Perspex box (20 × 12 × 14 cm) and exposed for 5 min to 7.5% citric acid aerosol delivered by an ultrasonic nebulizer (G.B. Elbisonic, Bielin Milan, Italy) with an output of 0.5 ml/min and particle diameter of 0.5–0.6 μm. During exposure to the aerosol, the number of coughs was counted.

2.3. Experiment 2: electrical stimulation of the superior laryngeal nerve

The method used was that described by Domenjoz (1952). Animals were lightly anesthetized with pentobarbital sodium (30 mg/kg i.p.). After an incision was made in the cervical midline under local anesthesia with lidocaine spray (Xylocaine® spray, Fujisawa Pharmaceutical, Osaka, Japan), a Y-shaped cannula was inserted into the trachea. The jugular vein was cannulated with a polyethylene venous catheter (4 Fr, Atom, Tokyo, Japan) for drug administration. The left or right superior laryngeal nerve was carefully dissected free from surrounding connective tissue and cut as peripherally to the site as possible. The central stump of the superior laryngeal nerve was sucked into a glass microelectrode previously filled with Ringer's solution (0.15 M NaCl, 4.02 mM KCl and 2.97 mM $CaCl_2$). Coughing was induced by afferent electrical stimulation of the superior laryngeal nerve by using an isolator (SS-201J, Nihon Kohden, Tokyo, Japan) attached to a stimulator (SEN-7203, Nihon Kohden). The following stimulation parameters were used: duration of individual square waves, 1 ms; wave train frequency, 5 Hz; voltage, 0.5–4 V; total duration of wave train, 10–20 s. The drug was administered after the minimum voltage and total duration required to elicit reproducible coughing were determined by stimulation at 5-min intervals. Electrical stimuli with the same voltage and total duration threshold were also given 5, 10, 15 and 30 min after dosing. Changes in intratracheal pressure caused by coughing were measured by using a differential pressure transducer (Model DP 45, Validyne, Northridge, CA, USA) connected to the tracheal cannula. Signals obtained from the differential pressure transducer were amplified by a carrier amplifier (Model 11-G4113-01, Gould, Valley View, OH, USA) and recorded on a pen recorder (U-228, Unique Medical, Tokyo, Japan). Rectal temperature was maintained at 37°C throughout the experimental period.

2.4. Drugs

In experiment 1, moguisteine (Boehringer Mannheim Italia, Monza, Italy) was dissolved in dimethyl sulphoxide (Carlo Erba, Milan, Italy) and diluted with distilled water (1:1, v/v). Citric acid (Carlo Erba) was dissolved in distilled water. In experiment 2, for i.v. administration moguisteine was dissolved in ethanol (Nacalai Tesque, Kyoto, Japan) plus polyethyleneglycol #400 (Nacalai Tesque) and diluted with 2.6% NaCl (10:54:36, v/v). For i.c.v. injection moguisteine was dissolved in dimethyl sulphoxide (Wako, Osaka, Japan) and diluted with saline (6:94, v/v). Codeine phosphate (Shionogi, Osaka, Japan) and naloxone hydrochloride (Sigma, St. Louis, MO, USA) were dissolved in saline. The doses described in this report refer to the free base.

Table 1
Antitussive effect of i.v. administration of moguisteine on the cough reflex induced by inhalation of citric acid aerosol in conscious guinea pigs

Treatment	No. of coughs	% reduction vs. control	ED_{50} (95% CL) (mg/kg)
Control (vehicle)	15.75 ± 2.66	–	
Moguisteine 2.5 mg/kg	10.88 ± 3.44	31	5.5
Moguisteine 5 mg/kg	8.25 ± 3.15	48	(3.8–8.6)
Moguisteine 10 mg/kg	5.50 ± 2.62	65	

Data are expressed as the means ± S.E.M. for eight animals. Moguisteine was administered i.v. 5 min before inhalation of 7.5% citric acid aerosol for 5 min. Control animals received vehicle (dimethyl sulphoxide/distilled water) in a volume of 1 ml/kg i.v. The antitussive effect of the drug was evaluated during inhalation of the aerosol. The ED_{50} value with the 95% CL was calculated with the Probit method.

2.5. Drug administration

2.5.1. I.v. injection

Moguisteine (2.5–20 mg/kg) and codeine (0.5–4 mg/kg) were administered i.v. Control animals were given vehicle in a volume of 1 ml/kg i.v. Naloxone (2 mg/kg) was administered i.p. 10 min before treatment with codeine.

2.5.2. I.c.v. injection

Under pentobarbital sodium (30 mg/kg i.p.) anesthesia, the animal's head was fixed in a stereotaxic apparatus (Mouter diameterel 900, David Kopf Instruments, Tujunga, CA, USA). A midline incision was made from a point just posterior to the eyes to about 3 cm caudal, and the overlying connective tissue was removed to expose the skull. A small hole (diameter, about 2 mm) was opened perpendicularly to the skull, −2.5 or −3.0 mm anterior and 2.5 or 3.0 mm lateral to the bregma by using a dental drill (Minitor-8, Kanto Kiki, Tokyo, Japan). A stainless steel guide cannula (outer diameter, 1.25 mm; length, 1.5 cm) was then slowly and vertically lowered to a depth of 2.5 or 3.0 mm from the dura into the brain. The guide cannula was then held in place by dental cement (Fuji I, GC, Tokyo, Japan) with a small anchor screw. Thereafter, the animals were prepared for afferent electrical stimulation of the superior laryngeal nerve by the method de-

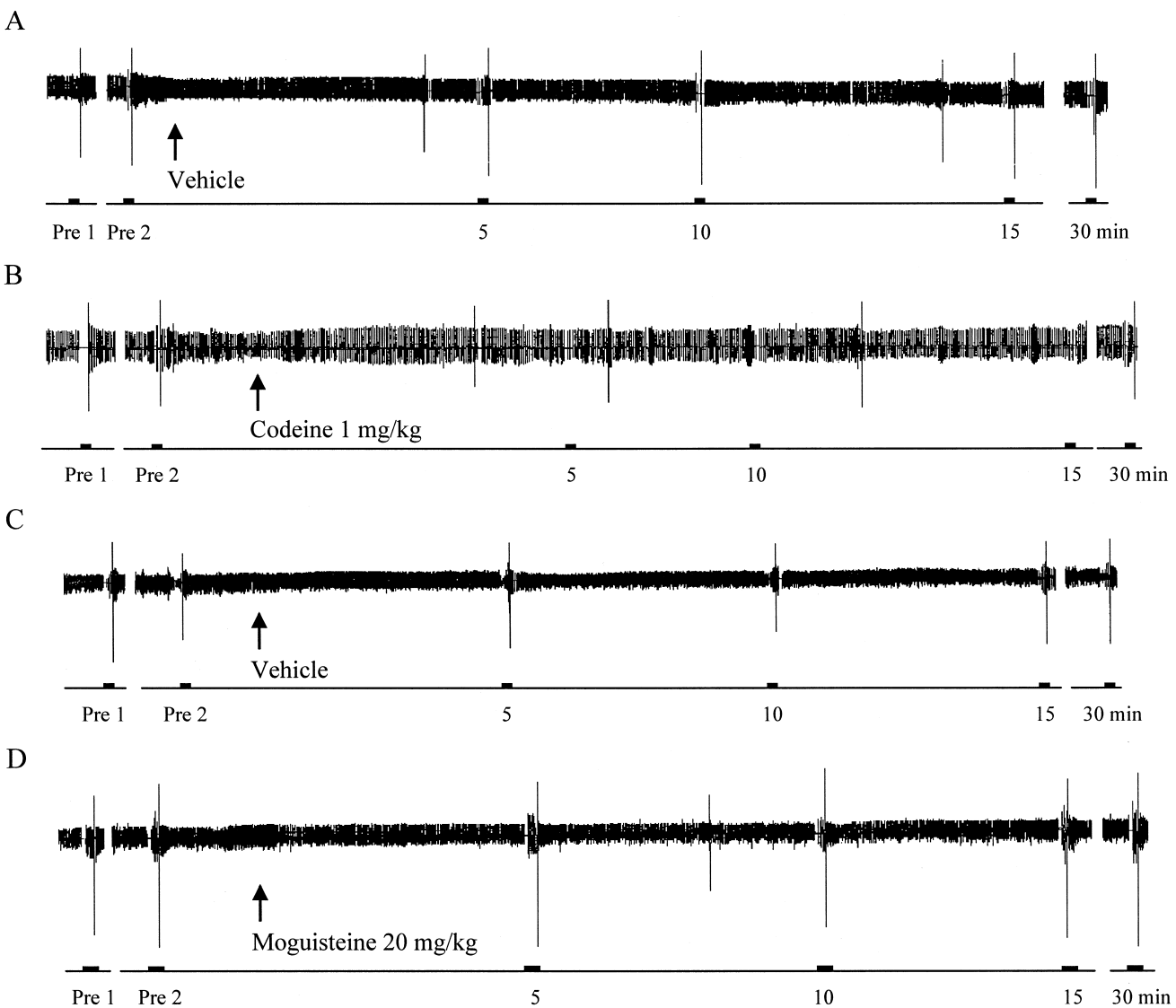

Fig. 1. Typical recordings of the cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve before and after i.v. administration of codeine and moguisteine in anesthetized guinea pigs. The cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve is represented as a pronounced increase in intratracheal pressure. Both codeine and moguisteine were administered i.v. when the cough reflex was reproducibly provoked by stimulating the superior laryngeal nerve twice at 5-min intervals, with a constant stimulation threshold (duration of individual square waves, 1 ms; wave train frequency, 5 Hz; voltage, 1.0, 1.5 or 2.5 V; total duration of wave train, 10 or 15 s) during the pretreatment period. Control animals received each vehicle in a volume of 1 ml/kg i.v. The same electrical stimuli were also applied to the superior laryngeal nerve at 5, 10, 15 and 30 min after dosing. (A) Vehicle (saline)-treated control, (B) codeine at 1 mg/kg, (C) vehicle (ethanol/polyethyleneglycol #400/2.6% NaCl)-treated control, (D) moguisteine at 20 mg/kg.

scribed above. A stainless steel injector (outer diameter, 0.9 mm; length, 2 mm longer than the guide cannula) connected to a micro-syringe (100 μl) and polyethylene tubing (outer diameter, 0.9 mm; length, about 50 cm) was used for i.c.v. injection. Moguisteine (20 μg) and codeine (5–20 μg) were injected by using an infusion pump (Model 100, KD Scientific, Boston, MA, USA) at a flow rate of 5 μl/min. Control animals received vehicle in a volume of 20 μl. At the end of the experiment, 20 μl of 6% methylene blue solution was injected through the i.c.v. cannula to check that the injection of drug had been correct.

2.6. Data analysis

In experiment 1, the antitussive effect of the drug was expressed as the percent reduction in coughing compared with that of the control group. In experiment 2, the antitussive activity of the drug was regarded as positive when no coughing was evoked by afferent electrical stimulation of the superior laryngeal nerve. In both experiments 1 and 2, the ED_{50} value with 95% confidence limits (CL) was calculated at 5 min after dosing by using the Probit method, because the maximal effect of the drug was expected at this time after i.v. and i.c.v. injections, when peak drug concentrations are attained in blood and brain, respectively.

3. Results

3.1. Experiment 1: antitussive effect of moguisteine on the cough reflex induced by inhalation of citric acid aerosol

Moguisteine (2.5–10 mg/kg i.v.) reduced the citric acid-induced cough reflex in a dose-dependent manner. The ED_{50} value with 95% CL in parentheses was 0.55 (3.8–8.6) mg/kg (Table 1).

3.2. Experiment 2: effects of moguisteine and codeine on the cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve

Fig. 1 shows typical recordings for the cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve before and after i.v. administration of codeine (B), moguisteine (D) and their vehicles (A and C, respectively) in anesthetized guinea pigs. As in the pretreatment periods (Fig. 1A–D), the cough reflex provoked by electrical stimulation of the superior laryngeal nerve was characterized by a pronounced increase in intratracheal pressure, consisting of a deep inflation followed by a large deflation. In both vehicle-treated control animals (Fig. 1A and C), the cough reflex was elicited by each stimulation 5–30 min after dosing, although a spontaneous cough reflex independent of stimulation of the superior laryngeal nerve was also occasionally observed. Codeine (1 mg/kg i.v.; Fig. 1B) consistently inhibited the cough reflex, apart from the occasional spontaneous outbursts, up to 15 min after dosing. Moguisteine (20 mg/kg i.v.; Fig. 1D) did not affect the cough reflex up to 30 min after dosing.

Table 2 shows the results 5 min after i.v. administration of codeine or moguisteine. Two animals did not show the cough reflex, i.e., were regarded as positive, even in the vehicle-treated control group. Codeine (0.5–4 mg/kg i.v.) inhibited the cough reflex in a dose-dependent manner. The ED_{50} value with 95% CL in parentheses was 0.91 (0.37–1.96) mg/kg. Pretreatment with naloxone (2 mg/kg i.p.) did not affect the cough reflex in the vehicle-treated control group: there was no change in the number of positive animals. The inhibitory effect of codeine (4 mg/kg i.v.) on the cough reflex was abolished by pretreatment with naloxone: the number of positive animals decreased from 8 to 2. Unlike codeine, moguisteine (4 and 20 mg/kg i.v.) hardly affected the cough reflex: the number of positive animals in both drug-treated groups was almost similar to that found in the vehicle-treated control group.

Table 3 shows the results 5 min after i.c.v. injection of codeine or moguisteine. A dose-dependent inhibition of the cough reflex was elicited by codeine (5–20 μg i.c.v.), although two positive animals were observed even in the vehicle-treated control group. The ED_{50} value with 95% CL in parentheses was 7.90 (3.14–28.77) μg. No inhibitory effect on the cough reflex was produced by mogu-

Table 2
Effects of i.v. administration of codeine and moguisteine on the cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve in anesthetized guinea pigs

Treatment	No. of animals regarded positive/ treated	ED_{50} (95% CL) (mg/kg)
Control (vehicle)	2/10	
Codeine 0.5 mg/kg	4/10	
Codeine 1 mg/kg	5/10	0.91
Codeine 2 mg/kg	7/10	(0.37–1.96)
Codeine 4 mg/kg	8/10	
Naloxone 2 mg/kg + vehicle	2/10	
Naloxone 2 mg/kg + codeine 4 mg/kgs2/10	2/10	
Control (vehicle)	3/10	
Moguisteine 4 mg/kg	3/10	
Moguisteine 20 mg/kg	4/10	

Codeine and moguisteine were administered i.v. Vehicle (saline or ethanol/polyethyleneglycol #400/2.6% NaCl) was administered in a volume of 1 ml/kg i.v. Naloxone was administered i.p. 10 min before treatment with codeine or vehicle. The antitussive effects of the drugs were evaluated at 5 min after dosing and were regarded as positive when no cough reflex was evoked by afferent electrical stimulation of the superior laryngeal nerve. The ED_{50} value with the 95% CL for the antitussive effect of codeine was calculated with the Probit method.

Table 3
Effects of i.c.v. injection of codeine and moguisteine on the cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve in anesthetized guinea pigs

Treatment	No. of animals regarded as positive/ treated	ED_{50} (95% CL) (μg)
Control (vehicle)	2/10	
Codein 5 μg	4/10	7.90
Codeine 10 μg	6/10	(3.14–28.77)
Codeine 20 μg	7/10	
Control (vehicle)	2/10	
Moguisteine 20 μg	2/10	

Codeine and moguisteine were injected i.c.v. by using an infusion pump at flow rate of 5 μl/min. Vehicle (saline or dimethyl sulphoxide/saline) was injected in a volume of 20 μl i.c.v. The antitussive effects of the drugs were evaluated at 5 min after dosing and were regarded as positive when no cough reflex was evoked by afferent electrical stimulation of the superior laryngeal nerve. The ED_{50} value with the 95% CL for the antitussive effect of codeine was calculated with the Probit method.

isteine (20 μg i.c.v.): the number of positive animals in this drug-injected group was equal to that found in the vehicle-treated control group.

4. Discussion

The experimental model of cough induced by afferent electrical stimulation of the superior laryngeal nerve was originally used with cats (Domenjoz, 1952). Since then, this cough model has been widely used for investigating the efficacy and the site of action of antitussive drugs (Rispat et al., 1976; Kase et al., 1983; Tarayre et al., 1983; Kamei et al., 1986a,b, 1987; Männistö et al., 1988). In the present study, we demonstrated in guinea pigs that a pronounced and reproducible cough reflex was provoked by electrically stimulating the superior laryngeal nerve. In cats, the antitussive effects of drugs are usually assessed by measurement of the frequency and/or the amplitude of the cough reflex. However, in guinea pigs, both variables were unsuitable as indices for quantitative analysis of the antitussive effects of drugs, because the cough reflex was only a single and not a repeated response, and its amplitude was not enhanced in proportion to the intensity of the stimulus. Accordingly, we evaluated the antitussive effects on the cough reflex of both moguisteine and codeine by summing the number of animals not showing the cough reflex, i.e. regarded as positive.

Using this cough model with guinea pigs, we found that both i.v. and i.c.v. injection of codeine dose dependently inhibited the cough reflex. Moreover, the antitussive effect of codeine (i.v.) was inhibited by pretreatment with naloxone. These results are in agreement with many previous reports indicating that the antitussive effect of codeine is mediated by central opioid mechanisms (Rispat et al., 1976; Kase, 1980; Kase et al., 1983; Tarayre et al., 1983; Männistö et al., 1988; Lavezzo et al., 1992; Gallico et al., 1994).

Moguisteine (2.5–10 mg/kg i.v.) dose dependently suppressed the cough reflex induced by inhalation of 7.5% citric acid aerosol in guinea pigs. However, the cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve was not inhibited by moguisteine even at a dose approximately 4-fold greater (20 mg/kg i.v.) than the ED_{50} value for its antitussive effect on the citric acid aerosol-induced cough reflex. Moreover, no inhibitory effect on the cough reflex was produced following i.c.v. injection of moguisteine despite the fact that we used the same dose (20 μg) at which codeine had a marked antitussive effect. This result is consistent with a previous report (Gallico et al., 1994) showing no antitussive effect of moguisteine (i.c.v.) on the cough reflex induced by electrical stimulation of the trachea in conscious guinea pigs. Taken together, the present findings suggest that the antitussive effect of moguisteine is mediated by peripheral rather than central mechanisms.

According to a recent study (Morikawa et al., 1997), the antitussive action of moguisteine is presumably mediated by inhibition of the activity of rapidly adapting receptors, rather than C-fiber receptors, since the drug did not appreciably affect the cardiovascular and respiratory responses to i.v. capsaicin, which presumably activated lung C-fiber receptors. However, levodropropizine, an antitussive drug that shares moguisteine's characteristics of peripheral action (Lavezzo et al., 1992) and non-opioid mechanism (Melillo et al., 1988), significantly reduced the responses to chemical stimulation of both pulmonary and non-pulmonary (broncheal) vagal afferent C-fibers. This finding suggests that the inhibitory effect of levodropropizine on these fibers accounts for its antitussive effect (Shams et al., 1996). However, there is no clear evidence about the other possible antitussive mechanisms of moguisteine, including actions at bronchial and pulmonary C-fiber receptors. Thus, further studies will be required to clarify the antitussive mechanisms of the drug.

In conclusion, to further demonstrate the peripheral antitussive properties of moguisteine, we studied the effect of the drug on the cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve in guinea pigs. The present findings suggest that the antitussive effect of moguisteine is mediated by peripheral mechanisms.

References

Charlier, R., Prost, M., Binon, F., Deltour, G., 1961. Etude pharmacologique d'un antitussif, le fumarate acide de phenethyl-1 (propyne-2-yl)-4 propionoxy-4 piperidine. Arch. Int. Pharmacodyn. 84, 306–327.

Domenjoz, R., 1952. Zur Auswertung hustenstillender Arzneimittel. Arch. Exp. Pathol. Pharmakol. 215, 19–24.

Gallico, L., Borghi, A., Dalla Rosa, C., Ceserani, R., Tognella, S., 1994. Moguisteine: a novel peripheral non-narcotic antitussive drug. Br. J. Pharmacol. 112, 795–800.

Kamei, J., Hosokawa, T., Yanaura, S., Hukuhara, T., 1986a. Effects of methysergide on the cough reflex. Jpn. J. Pharmacol. 42, 450–452.

Kamei, J., Hosokawa, T., Yanaura, S., Hukuhara, T., 1986b. Involvement of central serotonergic mechanisms in the cough reflex. Jpn. J. Pharmacol. 42, 531–538.

Kamei, J., Hukuhara, T., Kasuya, Y., 1987. Dopaminergic control of the cough reflex as demonstrated by the effects of apomorphine. Eur. J. Pharmacol. 141, 511–513.

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

Kase, Y., 1980. Antitussive agents and their sites of action. TIPS 5, 237–239.

Kase, Y., Kawaguchi, M., Takahama, K., Miyata, T., Hirotsu, I., Hitoshi, T., Okano, Y., 1983. Pharmacological studies on DL-glycine phosphate as an antitussive. Arzneim.-Forsch. Drug Res. 33, 936–946.

Lavezzo, A., Melillo, G., Clavenna, G., Omini, C., 1992. Peripheral site of action of levodropropizine in experimentally-induced cough: Role of sensory neuropeptides. Pulm. Pharmacol. 5, 143–147.

Männistö, P.T., Karttunen, P., Lahovaara, S., Nissinen, E., Davies, J.E., Algate, D.R., Baines, M.W., 1988. Antitussive action of the new anilide derivative vadocaine hydrochloride compared with codeine phosphate in four animal models. Arzneim.-Forsch. Drug Res. 38, 598–604.

Melillo, G., Malandrino, S., Rossoni, G., Caselli, G., Bestetti, A., Borsa, M., Tonon, G.C., Berti, F., 1988. General pharmacology of the new antitussive levodropropizine. Arzneim.-Forsch. Drug Res. 38, 1144–1150.

Morikawa, T., Gallico, L., Widdicombe, J., 1997. Actions of moguisteine on cough and pulmonary rapidly adapting receptor activity in the guinea pig. Pharmacol. Res. 35, 113–118.

Rispat, G., Burgi, H., Cosnier, D., Duchêne-Marullaz, P., Streichenberger, G., 1976. General pharmacological properties of a new non-opiate antitussive: Zipeprol (3024 CERM): I. Action on respiratory function and acute toxicity. Arzneim.-Forsch. Drug Res. 26, 523–530.

Sant'Ambrogio, G., Sant'Ambrogio, F.B., 1998. Action of moguisteine on the activity of tracheobronchial rapidly adapting receptors in the dog. Eur. Respir. J. 11, 339–344.

Shams, H., Daffonchio, L., Scheid, P., 1996. Effects of levodropropizine on vagal afferent C-fibres in the cat. Br. J. Pharmacol. 117, 853–858.

Tarayre, J.P., Vilain, P., Lauressergues, H., Cauquil, J., 1983. Pharmacological study of the antitussive and respiratory-analeptic properties of *N*-(2′-ethylpyrrolidino)diphenyl-acetamide hydrochloride (F 1459). Arzneim.-Forsch Drug Res. 33, 931–935.