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Antitussive effect of NS-398, a selective cyclooxygenase-2 inhibitor, in guinea pigs

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Abstract

Several reports have demonstrated that the number of capsaicin-induced coughs is increased in the presence of prostaglandins in the airway. Moreover, it has been reported that the expression of cyclooxygenase-2, which converts arachidonic acid to prostaglandins, was found in cultured human airway epithelial cells in the absence of inflammatory cytokine stimulation. Thus, it is possible that cyclooxygenase-2 inhibitor may produce an antitussive effect. To test this hypothesis, we investigated the effects of *N*-[2-(cyclohexyloxy)-4-nitrofenyl]-methane sulfonamide (NS-398), a selective cyclooxygenase-2 inhibitor, and 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl-pyrazole (SC-560), a selective cyclooxygenase-1 inhibitor, on capsaicin-induced coughs in guinea pigs. NS-398 (1–10 mg/kg, p.o.) dose-dependently and significantly reduced the number of capsaicin-induced coughs. In contrast, SC-560 (10 mg/kg, p.o.) did not reduce the number of capsaicin-induced coughs. The antitussive effect of NS-398 (10 mg/kg, p.o.) was not antagonized by pretreatment with methysergide (3 mg/kg, i.p.), a non-selective serotonin (5-HT) receptor antagonist, or glibenclamide (10 mg/kg, i.p.), an ATP-sensitive K^+ channel blocker. Furthermore, although NS-398 did not significantly affect the cough reflex induced by substance P (10^{-16} M), it significantly reduced the capsaicin-induced release of substance P in bronchoalveolar lavage fluid (BALF). The present findings clearly show that cyclooxygenase-2 inhibitor, but not cyclooxygenasez-1 inhibitor, has a potent antitussive effect. Furthermore, it is possible that the antitussive action of NS-398 does not depend on centrally acting mechanisms, since 5-HT receptors play an important role in the cough-depressant activities of centrally acting antitussive drugs. NS-398 may exert peripheral antitussive effects by inhibiting the release of substance P from capsaicin-sensitive afferent C-fibers in the airways. These results suggest that cyclooxygenase-2 inhibitors may have a therapeutic benefit in reducing coughs.

Keywords: Cyclooxygenase inhibitor; Cough reflex; Antitussive effect; NS-398; SC-560

1. Introduction

Coughing, which is a physiological defense mechanism for clearing foreign particles and excessive bronchial secretions from the airways, occurs in healthy people but is also a common symptom of a variety of respiratory diseases, such as asthma (Irwin et al., 1981). The cough reflex is triggered by stimulation of myelinated rapidly adapting receptors and unmyelinated C-fibers within the larynx, trachea and proximal bronchi (Widdicombe, 1995; Karlsson et al., 1988). Although it is well known that coughing plays a beneficial role in defending the host a persistent chronic cough can lead to physical exhaustion and is associated with significant morbidity (sleep loss, irritability, etc.) (O'Connell, 1998). Therefore, various agents have been used to try to suppress the cough response (Braman and Corrao, 1987).

Antitussive agents are generally classified as central or peripheral antitussives (Braman and Corrao, 1987). We previously suggested that centrally acting antitussive drugs,

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such as dihydrocodeine and dextromethorphan, exert their effects through the inhibition of glutaminergic synaptic transmission of the afferent input from sensory receptors of the airway by facilitating serotonergic mechanisms (Kamei, 1995, 1996). On the other hand, we also reported that peripheral antitussive drugs, such as moguisteine and Bakumondo-to, exert their effects through the activation of ATP-sensitive K^+ channels on afferent rapidly adapting receptors of the tracheobronchial tract (Morita and Kamei, 2000; Morita et al., 2002; Kamei et al., 2003). Thus, dihydrocodeine and dextromethorphan, which are central antitussive agents, penetrate the central nervous system following their systemic administration and reduce the responsiveness of the central neuronal elements that mediate cough. Peripheral antitussive agents, such as moguisteine and Bakumondo-to, exhibit little penetration of the central nervous system after systemic administration, but operate instead by reducing the responsiveness of the pulmonary vagal afferents that elicit cough. There is still a need for new antitussive agents that have no significant side effects while exerting powerful effects against coughs caused by upper respiratory tract infection and the persistent coughing caused by angiotensin-converting-enzyme (ACE) inhibitors and cough-variant asthma (Widdicombe, 1995).

Several reports have demonstrated that the number of capsaicin-induced coughs is increased in the presence of prostaglandins in the airway (Fujimura et al., 1995, Shinagawa et al., 2000). It is well known that cyclooxygenase is the rate-limiting enzyme for the conversion of arachidonic acid to prostanoids and exists in two isoforms. Cyclooxygenase-1 is constitutively expressed and is responsible for the basal production of prostanoids, whereas cyclooxygenase-2 is highly inducible by several stimuli, including cytokines, and is associated with inflammation. Sousa et al. (1997) found an increased expression of cyclooxygenase-2 in the epithelium and submucosa of asthmatic patients compared with control subjects. It has also been reported that the expression of cyclooxygenase-2 was detected in cultured human airway epithelial cells in the absence of inflammatory cytokine stimulation (Range et al., 2000).

Thus, it is possible that cyclooxygenase-2 inhibitors may have antitussive effects. To test this hypothesis, we investigated the effects of *N*-[2-(cyclohexyloxy)-4-nitrofenyl]-methane sulfonamide (NS-398), a selective cyclooxygenase-2 inhibitor, and 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl-pyrazole (SC-560), a selective cyclooxygenase-1 inhibitor, on capsaicin-induced coughs in guinea pigs. Furthermore, we also investigated the effects of glibenclamide, an ATP-sensitive potassium channel blocker, and methysergide, a serotonin receptor antagonist, to clarify the mechanisms of cyclooxygenase-induced antitussive effects underlying the capsaicin-induced cough reflex model in guinea pigs.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Tokyo Animal Laboratory, Tokyo, Japan) weighing about 300–350 g were used. The animals were housed in groups of four per cage under a 12-h light–dark cycle with food and water continuously available. These studies were carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals as adopted by the committee on care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Antitussive assay

The cough reflex was induced as previously described (Kamei et al., 1989; Kamei and Kasuya, 1992). Briefly, animals were exposed to a nebulized solution of capsaicin (30 μM) and/or substance P (10^{-16} M) under conscious and identical conditions using a body plethysmograph. Coughs were measured as airflow into or out of the chamber of the body plethysmograph with a pneumotachometer head and were recorded on a polygraph. The number of coughs produced during 7-min exposure period was counted. Capsaicin was dissolved to a concentration of 30 mg/ml in a 10% ethanol and 10% Tween 80 saline solution. The animals were exposed to capsaicin and/or substance P for 7 min at 60 min before the administration of antitussive drugs to determine the frequency of control coughs (Cc). The animals were also exposed to capsaicin for 7 min at 60 min after the administration of drugs. The number of coughs produced after administration of the antitussive drug (Ct) was compared to the number of control coughs (Cc). Control animals administrated by vehicle were subjected to the same handling.

2.3. Determination of SP content in bronchoalveolar lavage fluid (BALF)

BALF were collected from lung of guinea pigs which were deeply anaesthetized with sodium pentobarbital 50 mg/kg (i.p.). BALF was obtained by injecting 10 ml of ice-cold physiological saline (5 ml, twice) via a tracheal cannula. The collected BALF (approximately 7 ml) was centrifuged at 1500 rpm for 10 min. After supernatant fractions of the collected BALF were acidified to pH 3 with 1 N HCl, they were boiled for 15 min and passed through C18 Sep-Pak columns (Wako, Osaka, Japan) to elute substance P with 50% acetonitrile containing 0.1 % trifluoroacetic acid (Morioka et al., 2002). The extracted substance P fractions were lyophilized and were resolved into assay buffers (0.5 ml/fraction) for substance P enzyme linked immunosorbent assay (ELISA: Cayman Chemical, Ann

Arbor, MI, USA). The substance P levels in BALF were determined by ELISA (Arakawa et al., 1996). Diluted samples plotted on standard curves were run parallel with the respective standard curves. The detection limit of the assay for substance P was 0.2 pg/well.

2.4. Drugs

NS-398 and SC-560 were generously supplied by Tsumura, Tsukuba, Japan. Methysergide maleate was generously supplied by Novartis Pharma, Tokyo, Japan. Naloxone hydrochloride was purchased from Sigma, St. Louis, MO, USA. Naloxone (0.3 mg/kg, i.p.) or methysergide (3 mg/kg, i.p.) was injected 15 min before the administration of antitussive agents. Antitussive agents were suspended in 0.5% sodium carboxyl methyl-cellulose. All other drugs were dissolved in saline.

2.5. Statistics

Data are expressed as the means±S.E. To analyze the substance P levels, a one-way analysis of variance (ANOVA) followed by the Bonferroni/Dunn test was used for all experiments. The statistical significance of differences was assessed by the Mann–Whitney *U*-test to evaluate the antitussive effect. A level of probability of 0.05 or less was considered significant.

3. Results

3.1. Antitussive effects of NS-398 and SC-560

Exposure to capsaicin (30 μM) 60 min before and after the administration of vehicle induced 19.9±1.2 and 17.6±1.8 coughs, respectively (*n*=7) (Fig. 1). The effect of vehicle on the number of capsaicin-induced coughs was not significant. NS-398, a selective cyclooxygenase-2 inhibitor, at doses of 1, 3 and 10 mg/kg, p.o., dose-dependently inhibited the number of capsaicin-induced coughs when the antitussive effect was measured 60 min after administration (Fig. 1). However, SC-560, a selective cyclooxygenase-1 inhibitor, at doses of 10 and 30 mg/kg, p.o., did not significantly affect capsaicin-induced coughs when the antitussive effect was measured 60 min after administration (Fig. 1).

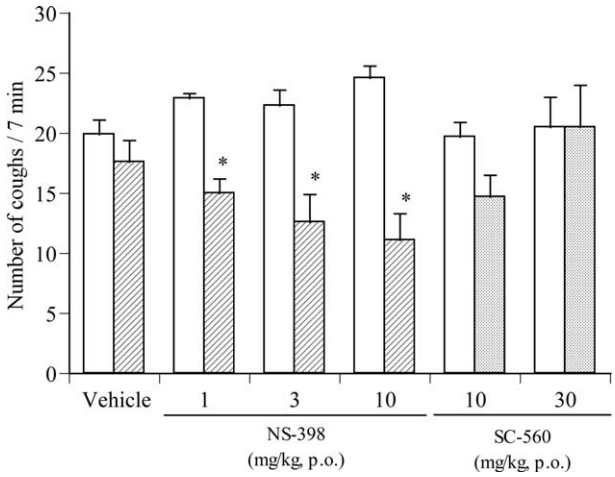

Fig. 1. Antitussive effects of NS-398 (1–10 mg/kg, p.o.) and SC-560 (10–30 mg/kg, p.o.). The antitussive effects of NS-398 and SC-560 were assessed 60 min after the administration of each drug. The effects of NS-398 and SC-560 on the number of capsaicin-induced coughs were determined. Each column represents the mean with S.E.M. (*n*=5–7). The number of coughs produced after antitussive drug administration (open column) was compared with the number of control coughs (hatted or dotted column). **P*<0.05 vs. number of control coughs.

3.2. Effects of methysergide and glibenclamide on the antitussive effect of NS-398

NS-398 at a dose of 10 mg/kg, p.o., had a potent antitussive effect in guinea pigs, and neither methysergide (3 mg/kg, i.p.), a selective 5-HT receptor antagonist, nor glibenclamide (10 mg/kg, i.p.), an ATP-sensitive K^+ channel blocker, significantly reduced the antitussive effect of NS-398 (Fig. 2). Glibenclamide (10 mg/kg, i.p.), by itself, had no significant effect on the number of capsaicin-induced coughs. (before glibenclamide, 20.7±1.3 coughs/7 min; after glibenclamide, 19.3±1.9 coughs/7 min, *n*=7). Methysergide (3 mg/kg, i.p.) also, by itself, had no significant effect on the number of capsaicin-induced coughs (before Methysergide, 20.4±0.7 coughs/7 min; after Methysergide, 18.9±0.7 coughs/7 min, *n*=7).

3.3. Effect of NS-398 (10 mg/kg, p.o.) on the substance P-induced coughs in guinea pigs

Exposure to substance P (10^{-16} M) 60 min before and after the injection of vehicle produced 9.7±1.6 and 8.8±2.0 coughs, respectively, and the vehicle did not have a significant effect on the number of substance P-induced coughs (Fig. 3). Similarly, since exposure to substance P (10^{-16} M) 60 min before and after the administration of NS-398 (10 mg/kg, p.o.) produced 10.8±0.9 and 9.0±1.4 coughs, NS-398 did not inhibit inhibited substance P (10^{-16} M)-induced coughs (Fig. 3).

3.4. Effect of NS-398 (10 mg/kg, p.o.) on the capsaicin-induced release of substance P in BALF

Exposure to capsaicin (30 μM for 5 min) 60 min after the injection of vehicle gave substance P levels of 5.2±0.96 ng/ml in guinea pig BALF (Fig. 4). However, as shown in Fig. 4, the substance P levels in BALF in NS-398 (10 mg/kg, p.o.)-treated guinea pigs (2.4±0.57 ng/ml)

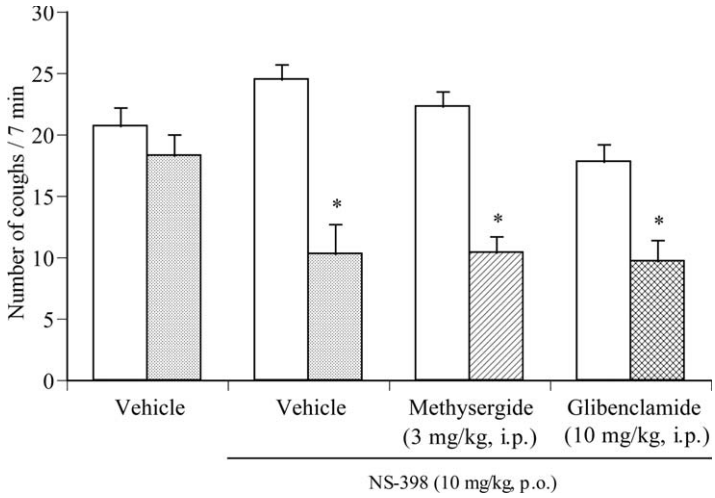

Fig. 2. Effects of methysergide and glibenclamide on the antitussive effect of NS-398 (10 mg/kg, p.o.). Methysergide (3 mg/kg) was injected i.p. 30 min before the administration of NS-398. Glibenclamide (10 mg/kg) was injected i.p. 5 min before the administration of NS-398. The antitussive effects of NS-398 were assessed 60 min after administration. Each column represents the mean with S.E. (n=6–8). The number of coughs produced after antitussive drug administration (open column) was compared with the number of control coughs (hatted or dotted column). *P<0.05 vs. number of control coughs.

were significantly lower than those in vehicle-treated guinea pig.

4. Discussion

In the present study, NS-398, a selective cyclooxygenase-2 inhibitor, but not SC-560 a selective cyclooxygenase-1 inhibitor, dose-dependently and significantly reduced the number of capsaicin-induced coughs. It is well known that cyclooxygenase-1 is constitutively expressed and is responsible for the basal production of prostanoids, whereas cyclooxygenase-2 is highly inducible by several stimuli, including cytokines, and is associated with inflammation. However, it has been reported that cyclooxygenase-2 was expressed in normal human respiratory epithelium and was not quantitatively unregulated in stable asthma (Demoly et al., 1997). Moreover, it has been reported that the expression of cyclooxygenase-2 was found in cultured human airway epithelial cells in the absence of inflammatory cytokine stimulation (Range et al., 2000). Several reports have demonstrated that the number of capsaicin-induced coughs is increased in the presence of prostanoids in the airway (Fujimura et al., 1995, Shinagawa et al., 2000). Furthermore, prostaglandins have been shown to influence the sensitivity of the cough reflex (Nichol et al., 1990; Stone et al., 1992; Lee et al., 2002). Thus, these results suggest that COX-2 inhibitor, but not COX-1 inhibitor, might play an important role in controlling the cough reflex.

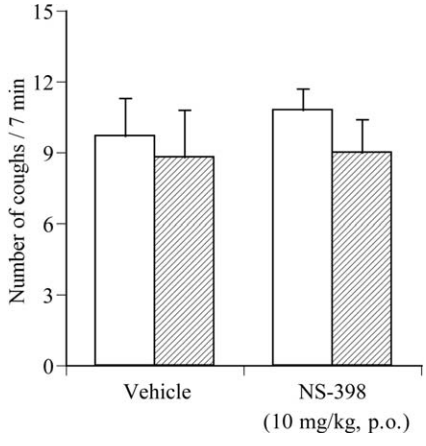

Fig. 3. Effect of NS-398 (10 mg/kg, p.o.) on substance P-induced coughs in guinea pigs. The antitussive effects of NS-398 were assessed 60 min after administration. The number of coughs produced after antitussive drug administration (open column) was compared with the number of control coughs (hatted or dotted column). Each column represents the mean with S.E.M. (n=7).

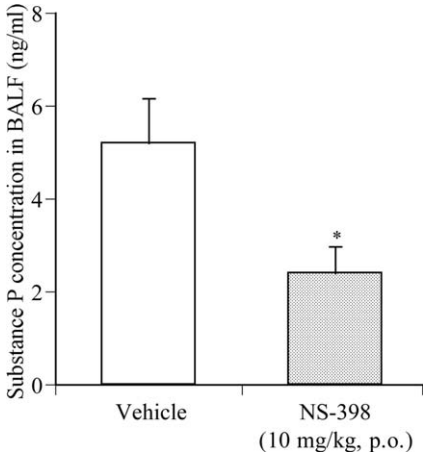

Fig. 4. Effect of NS-398 (10 mg/kg, p.o.) on the capsaicin-induced release of substance P in bronchoalveolar lavage fluid (BALF). After exposure to nebulized capsaicin (30 μM) for 7 min at 60 min after administration of the drug, BALF was obtained by injecting 10 ml of ice-cold physiological saline (5 ml, twice) via a tracheal cannula. Each column represents the mean with S.E.M. (n=7). *P<0.05 vs. vehicle-treated group.

Smith et al. (1998) demonstrated that oral administration of SC-560 in the doses of 10 mg/kg produced maximal inhibition of prostaglandin production. This result indicated that SC-560 in the doses that used in this study was enough to inhibit the cyclooxygenase-1. On the other hand, Ehrlich et al. (2004) also reported that similar influence was observed to the gastric mucosal using SC-560 (5 mg/kg) and NS-398 (1 mg/kg). Thus, this result indicate that the relative potencies of NS-398 are about five times higher than that of SC-560. In the present study, although NS-398 showed the significantly antitussive effect at the lower doses of 1 mg/kg, SC-560 did not show any significantly effect to capsaicin-induced coughs at the higher doses of 30 mg/kg. From these results, we proposed that cyclooxygenase-1 inhibitor might not participate in the regulation of the capsaicin-induced cough reflex in guinea pigs.

We previously demonstrated that a reduction in the level of serotonin (5-HT) in the whole brain decreased the potency of antitussive drugs that acted at the central nervous system, but not peripherally (Kamei et al., 1987). Neonatal treatment with 5,7-dihydroxytryptamine, which is sufficient to reduce whole brain levels of 5-HT to 19% of control levels, resulted in supersensitivity to the cough-depressant effect of dihydrocodeine (Kamei et al., 1988). Furthermore, the potentiation of the antitussive effect of dihydrocodeine observed in 5,7-dihydroxytryptamine-treated rats was abolished by pretreatment with methysergide, a 5-HT receptor antagonist. Therefore, the marked increase in the antitussive effect of dihydrocodeine might have been due to changes in the sensitivity of 5-HT receptors in the nucleus of the solitary tract. Thus, we suggested that 5-HT receptors in the nucleus of the solitary tract, which is one of the brain site which regulate the cough reflex, may play an important role in the cough-depressant activities of centrally acting, but not peripherally acting, antitussive drugs (Kamei et al., 1987, 1988, Kamei, 1996). On the other hand, we previously reported that the antitussive effect of subcutaneously administered or inhaled moguisteine, a peripherally acting non-narcotic antitussive drug (Gallico et al., 1994; Morikawa et al., 1997; Ishii et al., 1998; Sant'Ambrogio and Sant'Ambrogio, 1998), was reduced by pretreatment with glibenclamide, an ATP-sensitive K^+ channel blocker, in a dose-dependent manner (Morita and Kamei, 2000). However, pretreatment with glibenclamide had no effect on the antitussive effects of dihydrocodeine and dextromethorphan (Morita and Kamei, 2000). These results indicated that moguisteine, but not centrally acting antitussive drugs, may exert antitussive effects through the activation of ATP-sensitive K^+ channels. Based on these results, we proposed that ATP-sensitive K^+ channels might be involved in the antitussive effects of peripherally acting non-narcotic antitussive drugs (Morita and Kamei, 2000). In the present study, the antitussive effect of NS-398 was not significantly influenced by pretreatment with either methysergide or glibenclamide. These results suggest that NS-398 does not exert its antitussive effect through either the peripheral modulation of ATP-sensitive K^+ channels or the central activation of serotonergic systems.

It has been reported that neurokinin A and substance P are released upon the stimulation of airways C-fibers, and these neuropeptides have profound effects on rapidly adapting receptor's activity (Joad et al., 1997; Matsumoto et al., 1997). Studies in guinea pigs have confirmed that endogenously released neurokinins can activate airway rapidly adapting receptor, secondary to their effects on structural cells in the airway wall (Bergren, 1997, Morikawa et al., 1997). Bergren (1997) has shown that capsaicin markedly increases intrapulmonary rapidly adapting receptor activity in guinea pigs. Thus, the ability of capsaicin to increase rapidly adapting receptor activity secondary to the peripheral release of neurokinins from airway C-fiber nerve endings suggests that targeting the peripheral actions of neurokinins in the airways may induce cough reflexes initiated by C-fiber activation in guinea pigs. In the present study, the inhalation of substance P (10^{-16} M) initiated a repetitive cough reflex in guinea pigs. However, NS-398 did not affect the substance-P induced cough reflex. Although NS-398 did not reduce the number of SP-induced coughs, it significantly inhibited the capsaicin-induced release of substance P in BALF. Thus, it seems likely that cyclooxygenase-2 inhibitors may be involved in the release of substance P from capsaicin-sensitive sensory C-fibers.

The persistent activation of afferent C fibers leads to the release of several excitatory afferent transmitters, such as tachykinins, including substance P. Tachykinins result in depolarization and their release from afferent C fibers increases intracellular calcium levels at the C-fiber membrane, which in turn results in the activation of several intracellular enzymes, including phospholipase A2. Phospholipase A2 results in an increase in cytosolic arachidonic acid, which then enters the cyclooxygenase cascade and leads to the formation of a variety of prostaglandins that are released to the extracellular space. There is considerable evidence that endogenous prostaglandins facilitated the sensitization of capsaicin-sensitive sensory C-fibers (Takeuchi et al., 2003). Svensson and Yaksh (2002) also reported that prostaglandins can be increased the intracellular calcium by the mediated prostanoid receptors, and these effects can facilitate afferent transmitter release at the primary afferent C-fibers. Furthermore, several reports have demonstrated that the sensitivity of capsaicin-induced cough reflex was increased in the presence of prostaglandins in the airway (Fujimura et al., 1995, Shinagawa et al., 2000). On the other hand, it was also reported that the facilitation of substance P release from primary afferent neurons was regulated via the cyclooxygenase-2 (Morioka et al., 2002). Considering these findings, it is suggested that the endogenous prostaglandins which derived from cyclooxygenase-2 pathway may regulate the substance P release from the capsaicin-sensitive sensory C-fibers. In the present

study, NS-398 significantly inhibited the capsaicin-induced release of substance P in BALF. Thus, we considered that the inhibition of the substance P release by NS-398 might result in the regulation of endogenous prostaglandins by cyclooxgenase-2 inhibitor on the capsaicin-sensitive sensory C-fibers. However, further studies are necessary before these possibilities can be established with greater certainty.

In conclusion, the present results clearly show that cyclooxygenase-2 inhibitor, but not cyclooxygenase-1 inhibitor, has a potent antitussive effect. Furthermore, it is possible that the antitussive action of NS-398 dose not depend on centrally acting mechanisms, since 5-HT receptors play an important role in the cough-depressant activities of centrally acting antitussive drugs. NS-398 may exert peripheral antitussive effects through the inhibition of capsaicin-sensitive afferent C-fibers in airways. These results suggest that cyclooxygenase-2 inhibitors may have a therapeutic benefit in reducing coughs.

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