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Effects of epinastine hydrochloride on cholinergic neuro-effector transmission in canine tracheal smooth muscle

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Abstract

We determined the effects of epinastine hydrochloride, an anti-asthmatic drug, on cholinergic neuro-effector transmission in canine trachea. Isometric tension of tracheal strips was measured in the presence of indomethacin and propranolol. Epinastine (10^{-6} M) significantly suppressed the contraction evoked by electrical field stimulation, but had no effect on the acetylcholine-evoked contraction. An L-type Ca^{2+} channel blocker, nicardipine, did not suppress the electrical field stimulation-induced smooth muscle contraction and did not alter the inhibitory effect of epinastine. An N-type Ca^{2+} channel blocker, ω-conotoxin, suppressed the electrical field stimulation-induced contraction in a dose-dependent manner, and in a subthreshold/intermediate concentration abolished the inhibitory effect of epinastine. These findings indicate that epinastine exerts prejunctional inhibitory effects on airway smooth muscle of dogs, presumably by inhibiting acetylcholine release from vagal nerve terminals, and suggest that this effect is mediated by N-type Ca^{2+} channels.

Keywords: Epinastine; Cholinergic nerve; Acetylcholine; Neuro-effector transmission

1. Introduction

It is generally believed that the vagal nerves play an important role in the pathogenesis of bronchial asthma (Danser et al., 1987; Ito, 1988). Recently, it was demonstrated that acetylcholine release from vagal nerve terminals is regulated by many factors; for example, endogenous acetylcholine, prostaglandin E, and catecholamine inhibit transmitter release by activating muscarinic (Minette and Barnes, 1988) and prostaglandin E receptors (Inoue et al., 1984; Walters et al., 1984), and β-adrenoceptors (Danser et al., 1987; Ito, 1988), respectively. Other investigators reported that β-adrenergic receptors facilitate rather than inhibit acetylcholine release in the horse (Zhang et al., 1995, 1996). Epinastine (WAL 801 CL) has been reported to have potent inhibitory effects on histamine-induced contractions (Tasaka et al., 1991) and histamine release (Kamei et al., 1992a,b). In addition to these effects, it has been reported that epinastine modulates excitatory non-adrenergic non-cholinergic neuro-effector transmission in guinea pig airways (Dupont et al., 1996). However little is known about the effects of this agent on cholinergic neuro-effector transmission.

The present study was undertaken to elucidate the effect of epinastine on cholinergic neuro-effector transmission and to analyze the mechanism involved in this effect.

2. Materials and methods

2.1. General procedure

Six adult mongrel dogs of either sex (7–8 kg) were anesthetized with sodium pentobarbitone (30–40 mg/kg i.p.) and exsanguinated. Segments of cervical trachea were quickly resected. A dorsal strip of transversely running tracheal smooth muscle was separated from the cartilage and the mucosa and adventitial alveolar tissue were carefully removed. The tracheal smooth muscle was cut to a

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width of 1–1.5 mm and a length of about 5 mm for recording of mechanical responses. The preparation was bathed in a modified Krebs solution of the following ionic composition (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, $H_2PO_4^-$ 1.2, HCO_3^- 15.5 and glucose 11.5. The solution was aerated with 97% O_2 and 3% CO_2, and its pH was 7.3–7.4.

For measurement of mechanical responses, the tracheal tissue was mounted vertically in a 1-ml organ bath through which the test solution flowed continuously at 35–36°C at a rate of 3 ml/min. One end of the strip was tied by a fine silk thread to a mechanotransducer (Nihon Kohden, TB-612T) and the other to a hook at the bottom of the bath. The strips were set up with an initial tension of 0.1–0.2 g, which was found to be optimal in previous studies (Ito and Tajima, 1981), and mechanical activity was recorded with a pen recorder.

To produce neurogenic responses, electrical field stimulation was applied through a pair of Ag–AgCl plates fixed to both sides of the inner surface of the chamber, so that current pulse could pass horizontally across the tracheal smooth muscle tissue preparations. Repetitive stimuli were applied at 20 Hz,with a pulse of 0.8 ms duration and 20 V strength every 3 min using an electric stimulator (Nihon Kohden, SEN-7103).

Dog airway smooth muscle is innervated by both cholinergic and adrenergic nerves (Russel, 1980; Ito and Tajima, 1981), and noradrenaline released from sympathetic nerve terminals can activate prejunctional β-adrenoceptors to suppress cholinergic transmission (Danser et al., 1987). It has also been reported that the twitch contraction evoked by nerve stimulation decreases progressively in amplitude due to inhibition of transmitter release from vagal nerve terminals by endogenous prostaglandin E compounds (Russel, 1980; Walters et al., 1984; Shore et al., 1987). Therefore, the experiments with canine tracheal smooth muscle were performed in the presence of both propranolol (10^{-6} M) and indomethacin (10^{-5} M).

The contraction evoked by electrical field stimulation gradually increased and became stable after 20 min. The amplitude of the contraction was almost the same for at least 60 min. We observed the effects of drugs 20 min after during application, when the electrical field stimulation-induced contraction was stable.

To confirm that electrical field stimulation induces twitch contraction by stimulating the vagal nerve, we examined the effects of tetrodotoxin (10^{-7} M) and atropine (10^{-6} M) on the response to electrical field stimulation.

2.2. Study design 1

The effects of epinastine on neuro-effector transmission were examined. After the first contractile responses to electrical field stimulation or Acetylcholine were obtained, 10^{-8} M to 10^{-6} M of epinastine was added to the organ bath. 20 min after the application, the contractile responses to electrical field stimulation or acetylcholine were then measured again in the presence of epinastine.

2.3. Study design 2

To examine the role played by Ca^{2+} channels in mediating the effect of epinastine, we examined the effects of nicardipine (L-type Ca^{2+} channel blocker) and ω-conotoxin (N-type Ca^{2+} channel blocker). We measured contractile responses to electrical field stimulation before and after administration of nicardipine and ω-conotoxin.

In nerve terminals, acetylcholine release occurs during large inward Ca^{2+} currents through many simultaneously open Ca^{2+} channels (Stanley, 1993; Lemos et al., 1994). The vagal nerve contains Ca^{2+} channels which are mostly N-type (Lin and Lin-Shiau, 1997), but it is unclear whether it contains L-type Ca^{2+} channels (Raji and Nordmann, 1994). We therefore examined the effects of ω-conotoxin (Allen and Brown, 1993) and nicardipine (Cousins et al., 1993) on the response to electrical field stimulation. ω-Conotoxin (10^{-9} M) significantly suppressed the electrical field stimulation-induced contraction, and its EC_{50} was 10^{-11} M.

2.4. Drugs

Acetylcholine chloride, indomethacin, tetrodotoxin, propranolol and ω-conotoxin (Sigma, St. Louis, MO, USA), atropine sulphate (Daiichi Pharmaceutical, Tokyo, Japan), nicardipine (Yamanouchi Pharmaceutical, Tokyo, Japan) were used in the present study.

The drugs were added to the perfusion solution.

2.5. Statistics

Amplitudes of muscle contraction are standardized as percentages of the contraction evoked by 40 pulses in the control and expressed as arithmetic means and standard errors. Differences between means were tested for significance with Student's *t*-test. Findings of $P < 0.05$ were considered significant.

The contraction evoked by electrical field stimulation or acetylcholine was measured before and after application of epinastine hydrochloride, in the absence and presence of nicardipine and ω-conotoxin.

3. Results

The contraction evoked by electrical field stimulation was completely abolished by tetrodotoxin or atropine, indicating that electrical field stimulation stimulates the

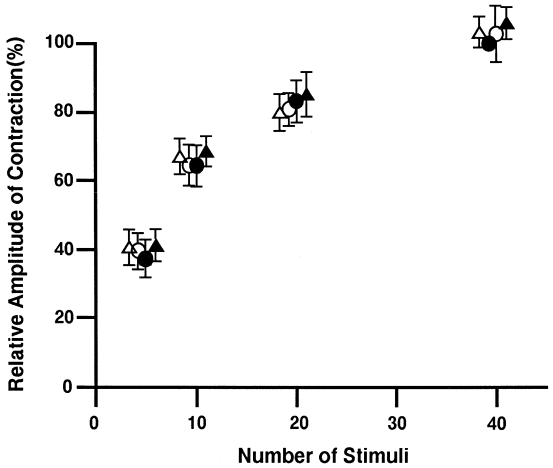

Fig. 1. The time-course of amplitude of contraction of dog tracheal smooth muscle evoked by electrical field stimulation. Intensity–response curve for electrical field stimulation was measured every 20 min. There was no significant difference in the amplitude of contraction between 4 consecutive intensity response curves (closed circle; control, closed triangle; 20 min after control, open triangle; 40 min after control, open circle; 60 min after control) ($n = 4$).

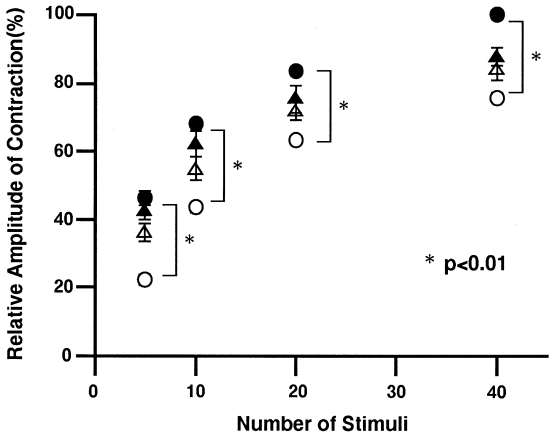

Fig. 3. Effects of epinastine on electrical field stimulation-induced contraction of dog tracheal smooth muscle. Relationship between relative amplitude of contraction and number of stimuli at 20 Hz, the amplitude of contraction evoked by 40 stimuli at 20 Hz with 0.8 ms pulse duration was taken to be 100%. Repetitive field stimulation with 5, 10, 20 or 40 stimuli at 20 Hz with 0.8 ms pulse duration was applied in the absence (closed circle) or presence (closed triangle: 10^{-8} M, open triangle: 10^{-7} M, open circle: 10^{-6} M) of epinastine, in the presence of indomethacin (10^{-5} M) and propranolol (10^{-6} M) ($n = 6$).

vagal nerve to contract smooth muscle. As shown in Fig. 1, the amplitude of contraction evoked by electrical field stimulation was not changed for at least for 60 min.

3.1. Effects of epinastine on electrical field stimulation- or acetylcholine-induced contraction in dog trachea

In Fig. 2, the effects of epinastine (10^{-6} M) on the electrical field stimulation-induced contraction and the acetylcholine-induced contraction in the presence of indomethacin and propranolol are illustrated. Epinastine (10^{-8} to 10^{-6} M) clearly suppressed the contraction evoked by electrical field stimulation, but had no effect on acetylcholie-evoked contraction. For example, in the dog trachea when 40 pulses at 20 Hz were applied every 3 min, epinastine suppressed the amplitude of twitch contractions to 70% of the control value.

The results are summarized in Fig. 3. The suppression of the electrical field stimulation-induced contraction in the presence of epinastine was statistically significant ($n = 6$, $P < 0.01$).

3.2. Effects of epinastine on acetylcholine-induced contraction of dog tracheal smooth muscle

To clarify the site of action of epinastine on tracheal smooth muscle, we examined the effects of epinastine on the sensitivity of the smooth muscle cells to acetylcholine.

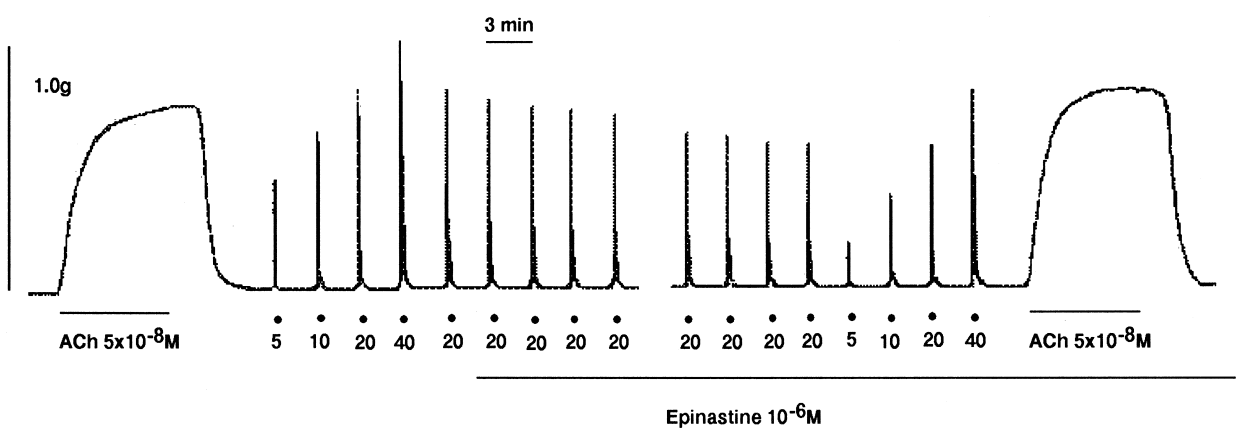

Fig. 2. Effects of epinastine (10^{-6} M) on electrical field stimulation-induced contraction and acetylcholine (5×10^{-8} M)-induced contraction of dog tracheal smooth muscle. Effects of epinastine (10^{-6} M) on electrical field stimulation-induced contraction and acetylcholine-induced contraction were measured in the presence of indomethacin (10^{-5} M) and propranolol (10^{-6} M). Repetitive field stimulation with 5, 10, 20 or 40 stimuli at 20 Hz with 0.8 ms pulse duration (dots) was applied in the absence or presence of epinastine in trachea.

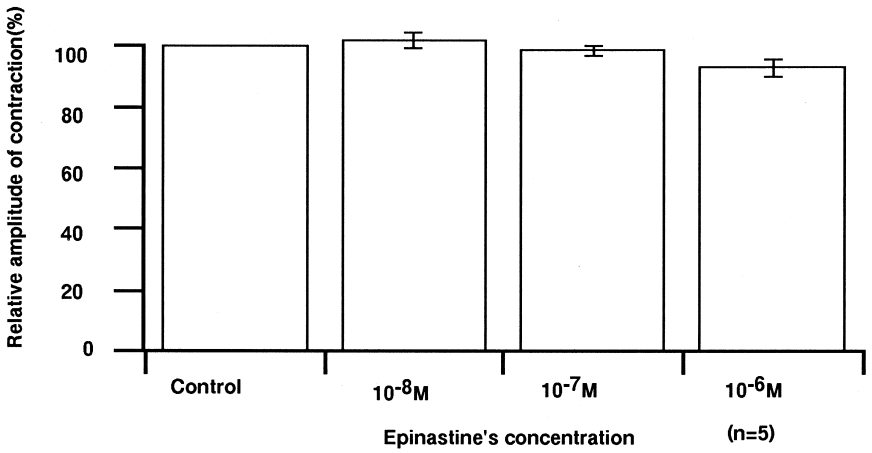

Fig. 4. Effects of epinastine on acetylcholine-induced contraction of dog tracheal smooth muscle. Effects of epinastine (10^{-6} M, 10^{-7} M, 10^{-8} M) on acetylcholine-induced contraction in the presence of indomethacin (10^{-5} M) and propranolol (10^{-6} M). Epinastine (10^{-6} M, 10^{-7} M, 10^{-8} M) had no statistically significant effect on acetylcholine-induced contraction of dog tracheal smooth muscle ($n = 6$).

For this purpose, the tension development induced by application of acetylcholine (5×10^{-8} M) was measured before and during application of epinastine (10^{-6} M, 10^{-7} M, 10^{-8} M). Contractions induced by acetylcholine were not changed before and during application of epinastine. The results of these experiments are summarized in Fig. 4 ($n = 6$).

3.3. Effects of epinastine on electrical field stimulation-induced contraction of dog tracheal smooth muscle treated with nicardipine

To clarify the roles played by different subtypes of Ca^{2+} channel in vagal nerve terminals, the effects of nicardipine, an L-type Ca^{2+} channel blocker (Lemos et al., 1994), on the action of epinastine were investigated. Nicardipine did not affect the electrical field stimulation-induced contraction at the doses of 10^{-9} to 5×10^{-7} M (data not shown).

Fig. 5 shows the effects of subthreshold doses of nicardipine (5×10^{-7} M) on the contraction of tracheal smooth muscle evoked by electrical field stimulation in the absence and presence of epinastine. The inhibitory effect of epinastine on the electrical field stimulation-induced smooth muscle contraction was not affected by nicardipine (5×10^{-7} M) ($n = 6$), suggesting that L-type Ca^{2+} channels do not play a role in meditating the inhibitory effect of epinastine on smooth muscle contraction.

3.4. Effects of ω-conotoxin on electrical field stimulation-induced contraction of dog tracheal smooth muscle

To clarify the subtypes of Ca^{2+} channel present in vagal nerve terminals, the effects of ω-conotoxin, an N-type Ca^{2+} channel blocker (Redman and Silinsky, 1995), on the action of electrical field stimulation-induced contraction were investigated (see Fig. 6). ω-Conotoxin suppressed the electrical field stimulation-induced contraction in a dose-dependent manner and abolished the electrical field stimulation-induced smooth muscle contraction at a concentration of 10^{-9} M. The inhibitory effect of epinastine on the electrical field stimulation-induced smooth muscle contraction was no longer significant in the presence of 10^{-11} M ω-conotoxin ($n = 5$), a concentration at

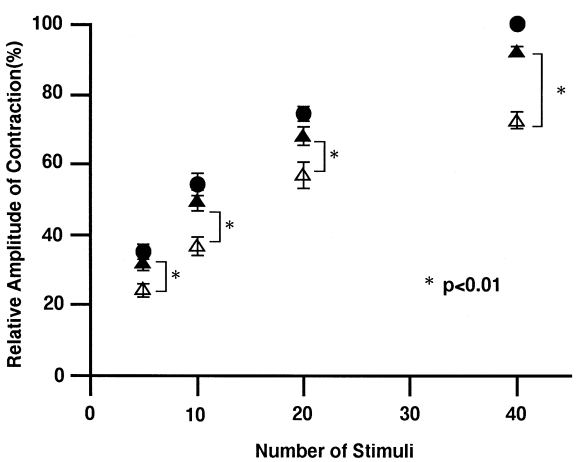

Fig. 5. Effects of epinastine on electrical field stimulation-induced contraction of dog tracheal smooth muscle pretreated with nicardipine. Relationship between relative amplitude of contraction and number of stimuli at 20 Hz, the amplitude of contraction evoked by 40 stimuli at 20 Hz with 0.8 ms pulse duration was taken to be 100%. Repetitive field stimulation with 5, 10, 20 or 40 stimuli at 20 Hz with 0.8 ms pulse duration was applied in the absence (closed circle)(closed triangle: nicardipine-pretreated) or presence (open triangle: 10^{-6} M) of epinastine, in the presence of indomethacin (10^{-5} M) and guanethidine (10^{-6} M). Nicardipine (5×10^{-7} M) did not decrease the inhibitory effect of epinastine on electrical field stimulation-induced smooth muscle contraction ($n = 6$).

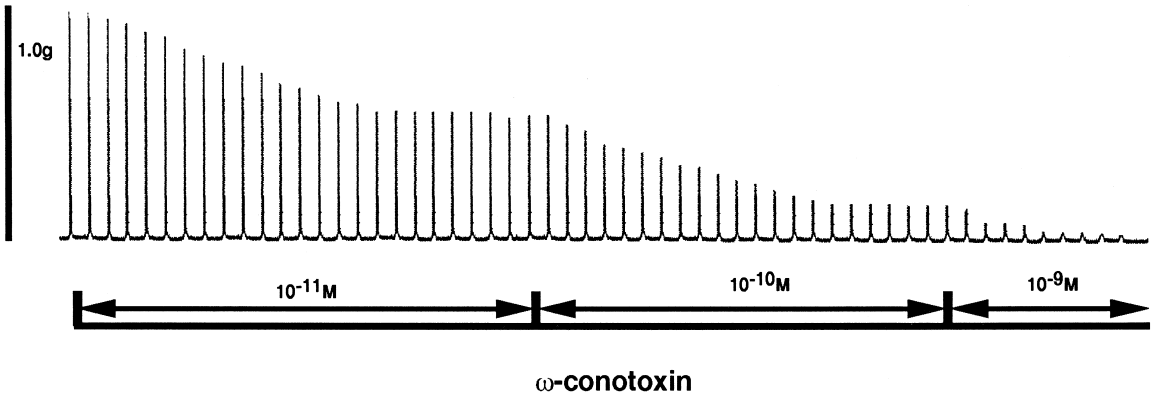

Fig. 6. Effects of ω-conotoxin on electrical field stimulation-induced contraction of dog tracheal smooth muscle. Effects of ω-conotoxin (10^{-11} M to 10^{-9} M) on electrical field stimulation-induced contraction in the presence of indomethacin (10^{-5} M) and propranolol (10^{-6} M). Repetitive field stimulation with 20 stimuli at 20 Hz with 0.8 ms pulse duration in trachea. ω-Conotoxin suppressed the electrical field stimulation-induced contraction in a dose-dependent manner at a concentration of 10^{-11} to 10^{-10} M, and completely abolished electrical field stimulation-induced smooth muscle contraction at a concentration of 10^{-9} M.

which alone it did not significantly affect the twitch contraction.

3.5. Effects of epinastine on electrical field stimulation-induced contraction of dog tracheal smooth muscle treated with ω-conotoxin

To clarify the subtypes of Ca^{2+} channel present in vagal nerve terminals, the effects of ω-conotoxin, an N-type Ca^{2+} channel blocker (Redman and Silinsky, 1995), on the action of epinastine were investigated (Fig. 7). The inhibitory effect of epinastine on the electrical field stimulation-induced smooth muscle contraction was not observed in the presence of 10^{-11} M ω-conotoxin ($n = 5$).

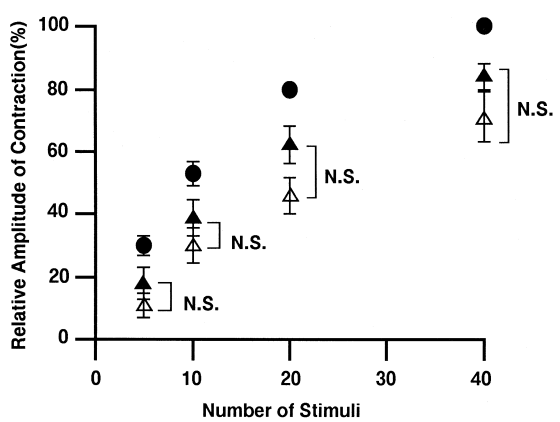

Fig. 7. Effects of epinastine on electrical field stimulation-induced contraction of dog tracheal smooth muscle pretreated with ω-conotoxin. Relationship between relative amplitude of contraction and number of stimuli at 20 Hz, the amplitude of contraction evoked by 40 stimuli at 20 Hz with 0.8 ms pulse duration was taken to be 100%. Repetitive field stimulation with 5, 10, 20 or 40 stimuli at 20 Hz with 0.8 ms pulse duration in the absence (closed circle)(closed triangle: ω-conotoxin-pretreated) or presence (open triangle: 10^{-6} M) of epinastine, in the presence of indomethacin (10^{-5} M) and guanethidine (10^{-6} M). ω-Conotoxin (10^{-11} M) did not decrease the inhibitory effect of epinastine on electrical field stimulation-induced smooth muscle contraction ($n = 5$).

This dose of ω-conotoxin did not affect the smooth muscle contraction.

4. Discussion

The findings obtained in this study demonstrate that epinastine inhibits excitatory cholinergic neuro-effector transmission in dog tracheal smooth muscle, in addition to its well-documented anti-histamine effects. Since epinastine significantly decreased the amplitude of the twitch contraction evoked by electrical field stimulation without affecting the contractions induced by acetylcholine, it probably inhibits smooth muscle contraction prejunctionally, presumably by suppressing acetylcholine release from vagal nerve terminals.

We did not measure the release of acetylcholine in the present study. Various endogenous substances are reported to modify the release of acetylcholine from vagus nerve terminals. Noradrenaline (Danser et al., 1987; Ito, 1988), prostaglandin E series (Inoue et al., 1984; Walters et al., 1984), and acetylcholine itself (Bloom et al., 1988; Minette and Barnes, 1988) suppress the release of acetylcholine from vagus nerve terminals. Prostaglandin D_2 (Tamaoki and Sekizawa, 1987) and substance P (Sekizawa et al., 1987; Aizawa et al., 1990) each increase the release of acetylcholine. The release of acetylcholine was inferred from the comparison of contractile responses to electrical field stimulation and those in response to exogenous acetylcholine. In a recent study acetylcholine was measured directly and the results obtained were similar to those of previous studies (Aas and Fonnum, 1986; Deckers et al., 1989; Kilbinger et al., 1991). It is strongly suggested that epinastine,which was used in this study, may suppress the release of acetylcholine from vagus nerve terminals.

It has been reported that epinastine acts as an antagonist of histamine H1 receptors in various tissues (50–100 mM in vitro, Kamei et al., 1992a,b; 20 mg/kg in vivo,

Tasaka et al., 1991), and that it inhibits bronchoconstriction induced by histamine or allergen challenge (0.1 mg/kg in vivo, Kamei et al., 1992a,b; 10 mg/kg in vivo, Meade et al., 1996; 10 mg/kg in vivo, Misawa and Kanai, 1991; 10 mg/kg in vivo, Misawa et al., 1991; 0.05 mg/kg in vivo, Tasaka et al., 1994). It has also been demonstrated that epinastine inhibits histamine release from rat peritoneal mast cells, and that this effect may be mediated in part by Ca^{2+}-calmodulin dependent process (50–100 mM in vitro, Kamei et al., 1992a,b).

The release of acetylcholine from nerve terminals is also a Ca^{2+}-dependent process. The precise mechanisms by which epinastine inhibits acetylcholine release from vagal nerve terminals are unknown. The L-type Ca^{2+} channel blocker nicardipine did not alter the inhibitory effect of epinastine on electrical field stimulation-induced smooth muscle contraction, suggesting that L-type Ca^{2+} channels do not play a role in this mechanism. By contrast, an inhibitory effect of epinastine on electrical field stimulation-induced smooth muscle contraction was not observed in the presence of the N-type Ca^{2+} channel blocker ω-conotoxin (10^{-11} M). Epinastine may block or modify N-type Ca^{2+} channels at the nerve terminal, thereby reducing acetylcholine release. Although ω-conotoxin and epinastine suppressed the smooth muscle contraction induced by electrical field stimulation, epinastine did not cause further suppression of the electrical field stimulation-induced contraction in the strips treated with ω-conotoxin. The lack of additive effects of ω-conotoxin and epinastine strongly suggests the contribution of an N-type Ca^{2+} channel-dependent mechanism in this model.

Orally administered epinastine inhibits histaminic activity with no effect on the central nervous system (Fugner et al., 1988; Schilling et al., 1990; Walther et al., 1990). In previous studies, epinastine had demonstrated multiple effects in addition to its anti-histaminic effect. These potent and long-lasting anti-allergic effects observed in animals or asthmatic subjects could be explained by its multiple actions (Fukuishi et al., 1995; Ki et al., 1996; Kohyama et al., 1997).

The clinical importance of this inhibitory effect of epinastine on smooth muscle in airway diseases is not yet understood. The autonomic nervous system plays a role in the development of airway hyperresponsiveness. Possible alterations which might lead to airway hyperresponsiveness include enhanced cholinergic and excitatory non-adrenergic non-cholinergic activity (Reed, 1974; Inoue et al., 1984; Said, 1987; Ward et al., 1994), or reduced β-adrenergic (Szentivanyi, 1968; Aizawa et al., 1991,) and inhibitory non-adrenergic non-cholinergic activity (Aizawa et al., 1982; Takahashi et al., 1995). Epinastine inhibited the release of acetylcholine from vagal nerve terminals in this study, which may be relevant to its therapeutic potential.

In summary, we demonstrated that epinastine suppresses transmitter release from vagal nerve terminals, presumably by inhibiting ω-conotoxin-sensitive N-type Ca^{2+} channels rather than nicardipine-sensitive L- type Ca^{2+} channels. Our findings suggest that the prejunctional effect of epinastine may contribute its potent bronchodilatative effects.

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