J. Pharm. Pharmacol. 1992, 44: 326–329
Received August 4, 1991

Non-opioid-dependent Inhibitory Action of Loperamide on Cholinergic Neurotransmission in Canine Isolated Bronchial Smooth Muscle

JUN TAMAOKI, SATSUKI HORII, MICHIO KATAYAMA, ISAO YAMAWAKI AND TAKAO TAKIZAWA

First Department of Medicine, Tokyo Women's Medical College, Tokyo 162, Japan

Abstract—The effect of loperamide on cholinergic neurotransmission in canine bronchial smooth muscle was studied under isometric conditions in-vitro. Addition of loperamide decreased contractile responses to electrical field stimulation in a dose-dependent fashion, the maximal decrease from the control response and the IC50 value being 65·4 ± 5·9% and 1·5 μM, respectively. In contrast, loperamide was without effect on the responses to exogenously administered acetylcholine. The inhibitory effect of loperamide was not altered by pre-incubation of tissues with propranol, 6-hydroxydopamine, bicuculline, or naloxone. These results suggest that loperamide attenuates the neurally mediated airway contraction probably by inhibiting acetylcholine release from cholinergic nerve terminals through a non-opioid-dependent mechanism.

Loperamide, a derivative of diphenoxylate and chemically related to pethidine, is commonly used as an antidiarrhoeal agent. The mechanism of action of this synthetic opioid agonist has been proposed to be inhibition of smooth muscle contraction, electrolyte and water transport (Sandhu et al 1983; Ooms et al 1984), and mucus secretion (Loeschke et al 1989) in the intestine. It has recently been shown that, as with the gastrointestinal tract, transepithelial chloride secretion in the respiratory tract is inhibited by loperamide through a non-opioid-dependent mechanism (Tamaoki et al 1990), thus raising the possibility that this drug could be of value in treating patients with excessive airway secretion such as chronic bronchitis and asthma.

In addition to airway hypersecretion, increased parasympathetic tone regulating airway calibre may be involved in the pathogenesis of asthma (Gold et al 1972; Mitchell et al 1986). Therefore, in the present study, to determine whether loperamide affects parasympathetic contractions of airway smooth muscle and, if so, what the mechanism of action is, we studied canine bronchial segments under isometric conditions in-vitro.

Materials and Methods

Tissue preparation

Mongrel dogs of either sex, 18–32 kg, were anaesthetized with intravenous sodium pentobarbitone (35 mg kg^{-1}) and both lungs were rapidly removed and immersed in Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 5·9, $CaCl_2$ 2·5, $MgSO_4$ 1·2, NaH_2PO_4 1·2, $NaHCO_3$ 25·5, and glucose 5·6. Bronchial segments were then dissected and mounted in organ chambers filled with 14 mL of Krebs-Henseleit solution maintained at 37°C and at pH 7·4 bubbled constantly with 95% O_2–5% CO_2. Tissues were connected to a force transducer (TB-652T, Nihon Kohden, Tokyo, Japan) for continuous recording of isometric tension by a pen recorder (WT-685G, Nihon Kohden). For transmural electrical field stimulation (EFS: biphasic pulse; pulse width, 0·5 ms; supramaximal voltage, 20 V for 20 s), each organ chamber was fitted with two rectangular platinum electrodes (4 × 30 mm) placed alongside the tissue.

After equilibration of tissues for 1 h while changing the bathing solution every 15 min, resting tension was adjusted to 4 g (Tamaoki et al 1987b). To avoid a possible release of prostaglandin E_2 which potently inhibits the release of acetylcholine from cholinergic nerve terminals in canine airways (Walters et al 1984), indomethacin (3×10^{-6} M) was present in the chambers throughout the experiments.

Effect of loperamide on parasympathetic contractions

To study the effect of loperamide on contractile responses of bronchial smooth muscle to EFS, we first obtained a frequency-response curve for EFS at increasing impulse frequencies (0·5–50 Hz) and, at 30 min after the addition of loperamide (10^{-5} M), we repeated the measurements. In control experiments, we examined the effect of the vehicle of loperamide (propylene glycol) alone on the responses to EFS. In evaluating the concentration-dependent effect, we measured the contractile responses to EFS at 5 Hz before and 30 min after the addition of loperamide (10^{-7}–3×10^{-5} M). The concentration of loperamide required to produce a half-maximal effect (IC50) was determined by linear regression analysis.

To determine whether the site of action of loperamide on bronchial smooth muscle is prejunctional or postjunctional, we also examined the effect of loperamide (10^{-5} M) on cumulative concentration-response curves for acetylcholine (10^{-8}–10^{-3} M) with the same time sequence for the measurements of the responses to EFS.

To assess the possible contribution of β-adrenergic actions, γ-aminobutyric acid $(GABA)_A$ receptors, and opioid receptors to the loperamide action, we examined the effects of preincubation of tissues with propranolol (10^{-5} M), a β-adrenergic receptor antagonist, 6-hydroxydopamine (10^{-3} M) that depletes noradrenaline in adrenergic neurones (Doggrell & Waldron 1982), the $GABA_A$ receptor antagonist bicucul-

Correspondence: J. Tamaoki, First Department of Medicine, Tokyo Women's Medical College, 8-1 Kawada-Cho, Shinjuku, Tokyo 162, Japan.

line (10^{-5} M) (Kaplita et al 1982), and the opioid receptor antagonist naloxone (10^{-5} M) on the loperamide (10^{-5} M)-induced decrease in contractile responses of bronchial segments to EFS at 5 Hz.

Drugs

The following drugs were used: loperamide hydrochloride (Dainippon Pharmaceutical, Tokyo, Japan; 10 mg dissolved in 0·5 mL propylene glycol for the stock solution and subsequently diluted with Krebs-Henseleit solution for the working solution), acetylcholine chloride, indomethacin, (±)-propranol hydrochloride, 6-hydroxydopamine, bicuculline, and naloxone (Sigma Chemicals, St Louis, MO).

Statistics

All values are expressed as means ± s.e.m. Statistical analysis was performed by analysis of variance or the Newman-Keuls multiple comparison test, and $P<0·05$ was considered significant.

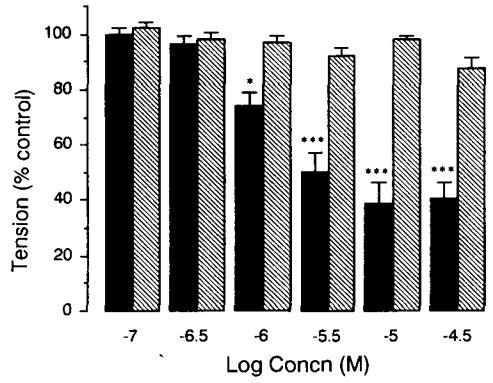

FIG. 2. Concentration-dependent effect of loperamide on contractile responses to electrical field stimulation at 5 Hz. Loperamide (■) or its vehicle propylene glycol (▨) was cumulatively added and the measurement was made 30 min after each addition. Responses are expressed as percent of control responses obtained before administration of a drug. Values are means ± s.e.m.; n = 9 for each column. $*P<0·05$, $***P<0·001$, compared with the corresponding baseline values.

Results

Addition of loperamide at 10^{-5} M did not alter the resting tone of canine bronchial smooth muscle. However, this agent markedly decreased the contractile responses to EFS at all stimulus frequencies tested (mean decrease, 40·7 ± 5·6% from control, $P<0·001$, n = 8), whereas the vehicle of loperamide solution, propylene glycol, alone was without effect (Fig. 1). Cumulative administration of loperamide inhibited the contractile responses to EFS at 5 Hz in a concentration-dependent fashion, the maximal decrease from the control response and the IC50 values being 65·4 ± 5·9% ($P<0·001$, n = 9) and 1·5 μM, respectively (Fig. 2).

In contrast to the effect on the EFS-induced contractions, contractile responses of bronchial smooth muscle to exogenously administered acetylcholine were not altered by 10^{-5} M loperamide (Fig. 3).

Preincubation of tissues with propranolol (10^{-5} M), 6-hydroxydopamine (10^{-3} M), bicuculline (10^{-5} M), or naloxone (10^{-5} M) by itself did not significantly alter the EFS (5 Hz)-induced muscle contraction. Moreover, neither of these pharmacologic blocking agents influenced the loperamide (10^{-5} M)-induced decrease in the contractile responses to EFS (Fig. 4).

Discussion

Our in-vitro studies demonstrate that loperamide dose-dependently decreases the neurally mediated contraction of canine bronchial smooth muscle probably through the inhibition of cholinergic neurotransmission.

It has been known that transmural electrical stimulation of the isolated canine airway, as performed in the present study,

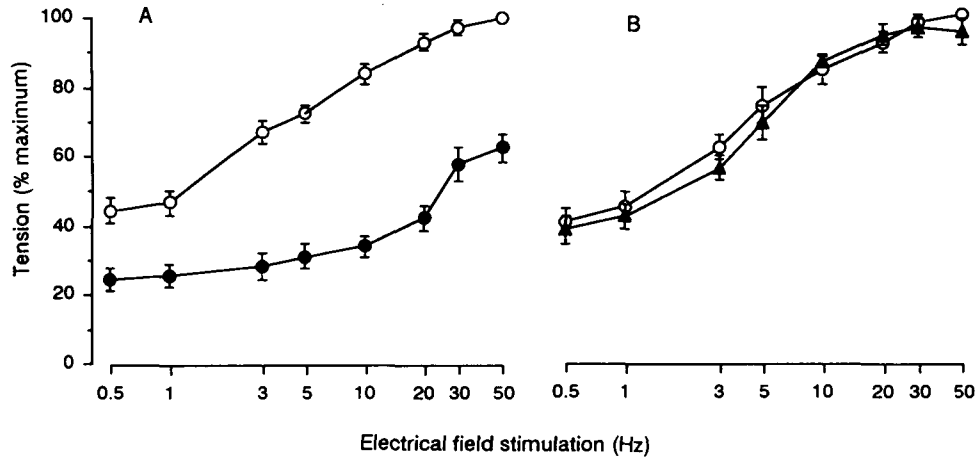

FIG. 1. Effects of loperamide (A) and its vehicle propylene glycol (B) on contractile responses of canine bronchial smooth muscle to electrical field stimulation at increasing impulse frequency. After obtaining control (○) responses to electrical field stimulation, loperamide (● 10^{-5} M) or diluted propylene glycol (▲) was added and, 30 min later, the measurements were repeated. Responses are expressed as percent of maximum control responses. Each point represents mean ± s.e.m.; n = 8.

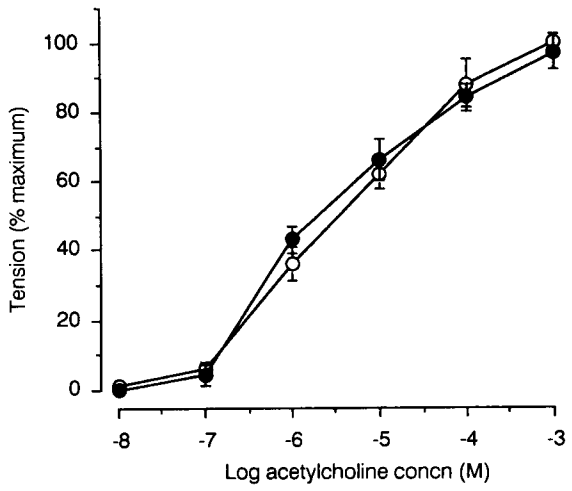

FIG. 3. Effect of loperamide on contractile responses to exogenous acetylcholine. After obtaining control (○) responses to acetylcholine (10^{-8}–10^{-3} M), loperamide (●, 10^{-5} M) was added and, 30 min later, the measurements were repeated. Responses are expressed as percentage of maximum control responses. Each point represents mean ± s.e.m.; n = 8.

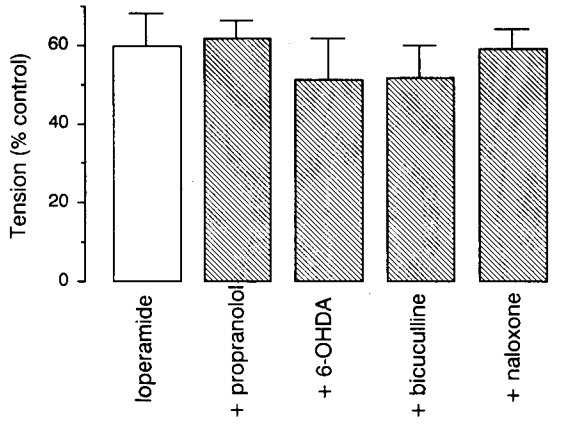

FIG. 4. Effects of propranolol (10^{-5} M), 6-hydroxydopamine (6-OHDA, 10^{-3} M), bicuculline (10^{-5} M) and naloxone (10^{-5} M) on the loperamide (10^{-5} M)-induced inhibition of contractile responses to electrical field stimulation at 5 Hz. Responses are expressed as percent of control responses obtained before loperamide addition. Values are means ± s.e.m.; n = 9 for each column.

stimulates postganglionic nerve fibres and elicits the excitatory response resulting from activation of vagal motor nerves and the inhibitory response resulting from activation of β-adrenergic mechanisms (Russell 1978). In our experiments, pretreatment of tissues with the β-adrenergic receptor antagonist propranolol or with 6-hydroxydopamine to deplete the content of noradrenaline in adrenergic nerve fibres (Doggrell & Waldron 1982) did not influence the inhibitory action of loperamide on the EFS-induced muscle contraction. Thus, the effect of loperamide may not be associated with β-adrenergic mechanisms but rather be attributable to modification of cholinergic neural mechanisms and/or from an alteration of the sensitivity of smooth muscle cells to released acetylcholine. To determine which component was responsible, we compared the effect of this agent on airway contraction induced by EFS with that induced by exogenously administered acetylcholine and found that, in contrast to the effect on the EFS-induced contraction, loperamide did not alter the contractile responses to acetylcholine. This finding suggests that the loperamide-induced inhibition may not be due to concomitant alterations in smooth muscle functions such as decreased muscarinic receptor binding or increased degradation of acetylcholine. Therefore, loperamide seems to act at prejunctional vagal nerve fibres and may inhibit the evoked release of acetylcholine from cholinergic nerve terminals. To confirm this, further studies on the direct measurement of acetylcholine release might be needed (Martin & Collier 1986). In addition, the fact that loperamide reduced airway contraction through specific inhibition of cholinergic nerve function without affecting adrenergic neural mechanisms or smooth muscle cell functions may exclude the possible local anaesthetic action of this drug at concentrations of less than 10^{-5} M.

It has been shown that canine airway smooth muscle synthesizes and releases prostaglandin E_2, which can inhibit the release of acetylcholine (Walters et al 1984). However, because the cyclo-oxygenase inhibitor indomethacin was present throughout the experiments, contribution of this prostanoid to the effect of loperamide seems unlikely. In addition, activation of prejunctional $GABA_A$ receptors and opioid receptors has been shown to depress acetylcholine release from the airway vagal motor pathway (Russell & Simons 1985; Tamaoki et al 1987a). However, contributions of these mechanisms are also unlikely because pretreatment of tissues with the $GABA_A$ receptor antagonist bicuculline (Kaplita et al 1982) and the opioid receptor antagonist naloxone did not influence the effect of loperamide. The latter finding is in agreement with previous demonstrations that the action of loperamide in the intestine is not dependent on opioid receptors (Yagasaki et al 1978; Balkovetz et al 1987).

Loperamide has been proposed to act as an antidiarrhoeal agent through inhibition of colonic smooth muscle contractions and water secretion. As with our present study, Burleigh (1988) reported that loperamide depressed cholinergic nerve function in human isolated colon, an effect that was mediated by a non-opioid receptor process. Although the exact mechanism of this action is uncertain, the inhibition of acetylcholine release by this agent may be due to calmodulin inhibition (Zavecz et al 1982) as this calcium binding protein is necessary for the release of neurotransmitters (De Lorenzo 1982). In contrast, we did not detect an effect of loperamide on β-adrenergic functions in the present study. The reason for this is unknown, but one possible explanation would be that the binding sites for loperamide could be specifically localized on cholinergic nerve fibres.

Apart from its antidiarrhoeal property, loperamide has recently been shown to inhibit Cl secretion across airway epithelium (Tamaoki et al 1990), suggesting that this agent might reduce airway secretions. Our present study may raise the possibility that loperamide could also be useful in treating patients with airway hyperreactivity by inhibiting cholinergic tone of airway smooth muscle.

References

Balkovetz, D. F., Miyamoto, Y., Tiruppathi, C., Mahesh, V. B., Leibach, F. H., Ganapathy, V. (1987) Inhibition of brush-border membrane Na^+-H^+ exchanger by loperamide. J. Pharmacol. Exp. Ther. 243: 150–154

Burleigh, D. E. (1988) Opioid and non-opioid actions of loperamide on cholinergic nerve function in human isolated colon. Eur. J. Pharmacol. 152: 39–46

De Lorenzo, R. J. (1982) Calmodulin in neurotransmitter release and synaptic function. Fed. Proc. 41: 2265–2272

Doggrell, S. A., Waldron, J. B. (1982) The effect of 6-OHDA or (−)-noradrenaline treatment in-vitro on noradrenergic transmission in the rat anococcygeus muscle. J. Auton. Pharmacol. 2: 231–240

Gold, W. M., Kessler, G.-F., Yu, D. Y. C. (1972) Role of vagus nerves in experimental asthma in allergic dogs. J. Appl. Physiol. 33: 719–725

Kaplita, P. V., Waters, D. H., Triggle, D. J. (1982) GABA action in guinea pig ileal myenteric plexus. Eur. J. Pharmacol. 79: 43–51

Loeschke, K., Schmid, T., Farack, U. M. (1989) Inhibition by loperamide of mucus secretion in the rat colon in vivo. Ibid. 170: 41–46

Martin, J. G., Collier, B. (1986) Acetylcholine release from canine isolated airway is not mediated by norepinephrine. J. Appl. Physiol. 61: 1025–1030

Mitchell, R. W., Antonissen, L. A., Kepron, W., Kroeger, E. A., Stephens, N. L. (1986) Effect of atropine on the hyperresponsiveness of ragweed-sensitized canine tracheal smooth muscle. J. Pharmacol. Exp. Ther. 236: 803–809

Ooms, L. A., Degryse, A. D., Janssen, P. A. J. (1984) Mechanism of action of loperamide. Scand. J. Gastroenterol. 19 (Suppl. 96): 145–155

Russell, J. A. (1978) Responses of isolated canine airways to electric stimulation and acetylcholine. J. Appl. Physiol. 45: 690–698

Russell, J. A., Simons, E. J. (1985) Modulation of cholinergic neurotransmission in airways by enkephalin. Ibid. 58: 853–858

Sandhu, B. K., Milla, P. J., Harries, J. T. (1983) Mechanisms of action of loperamide. Scand. J. Gastroenterol. 18 (Suppl. 84): 85–92

Tamaoki, J., Graf, P. D., Nadel, J. A. (1987a) Effect of γ-aminobutyric acid on neurally mediated contraction of guinea pig trachealis smooth muscle. J. Pharmacol. Exp. Ther. 243: 86–90

Tamaoki, J., Sekizawa, K., Graf, P. D., Nadel, J. A. (1987b) Cholinergic neuromodulation by prostaglandin D_2 in canine airway smooth muscle. J. Appl. Physiol. 63: 1396–1400

Tamaoki, J., Sakai, N., Isono, K., Takizawa, T. (1990) Inhibition by loperamide of chloride transport across canine cultured tracheal epithelium. Eur. J. Pharmacol. 190: 255–258

Walters, E. H., O'Byrne, P. M., Fabbri, L. M., Graf, P. D., Holtzman, M. J., Nadel, J. A. (1984) Control of neurotransmission by prostaglandins in canine trachealis smooth muscle. J. Appl. Physiol. 57: 129–134

Yagasaki, O., Suzuki, H., Shoji, Y. (1978) Effect of loperamide on acetylcholine and prostaglandin release from isolated guinea pig ileum. Jpn. J. Pharmacol. 28: 873–882

Zavecz, J. H., Jackson, T. E., Limp, G. L., Yellin, T. O. (1982) Relationship between anti-diarrhoeal activity and binding to calmodulin. Eur. J. Pharmacol. 78: 375–377