Contribution of External Ca\(^{2+}\) to the Modulation by Prostaglandin E\(_2\) of the Release of Acetylcholine from the Myenteric Plexus of Guinea Pig Ileum

Tadayoshi TAKEUCHI, Mitsuru SHIMIZU*, Miho OKUDA** and Osamu YAGASAKI

Department of Veterinary Pharmacology, Faculty of Agriculture, University of Osaka Prefecture, Sakai 591, Japan

Accepted December 15, 1988

Abstract—The effects of external concentration of Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_o\)) on the modulation by indomethacin (IND) or prostaglandin E\(_2\) (PGE\(_2\)) of the evoked release of acetylcholine (ACh) were investigated in the myenteric plexus of guinea pig ileum. An increase in the [Ca\(^{2+}\)]\(_o\) from 0.45 to 3.6 mM depressed both the spontaneous and the nicotine-induced release of ACh, while the releases of ACh induced by electrical field stimulation (EFS) or by high K\(^+\) were augmented. IND and PGE\(_2\) did not modify the release of ACh induced by EFS and high K\(^+\), at any given [Ca\(^{2+}\)]\(_o\), tested. The nicotine-induced release of ACh was significantly inhibited by IND with no relation to [Ca\(^{2+}\)]\(_o\), and these inhibitory effects of IND were prevented by PGE\(_2\). At 0.45 mM [Ca\(^{2+}\)]\(_o\), the extent of the recovery by PGE\(_2\) was less potent than those at other [Ca\(^{2+}\)]\(_o\). IND inhibited the spontaneous release of ACh at lower [Ca\(^{2+}\)]\(_o\), and such inhibition was not observed in the presence of PGE\(_2\). Thus, the modulatory actions of PGE\(_2\) on the spontaneous and the nicotine-induced release of ACh may depend partially on [Ca\(^{2+}\)]\(_o\).

Endogenous prostaglandins (PGs) have been reported to play roles in maintaining the excitability of the myenteric neurons of guinea pig ileum in response to nicotine (1, 2). However, the mechanism by which endogenous PGs modulate the nicotine-induced release of ACh remains to be elucidated. In some tissues, PGs increase the uptake of \(^{45}\)Ca (3, 4) and Ca antagonists interfere with the actions of PGs (4, 5). In addition, PGs increase the permeability of the cell membrane to Ca\(^{2+}\) in smooth muscle (6) and release Ca\(^{2+}\) from the mitochondria of liver (7) and blood vessels (8), the actions being similar to Ca ionophore. In the myenteric plexus of the guinea pig ileum, the release of ACh is triggered by a rise in intracellular Ca\(^{2+}\), which is mainly due to an increase in the influx of Ca\(^{2+}\) (9). It has been demonstrated that agents that increase the rate of entry of Ca\(^{2+}\), such as Ca ionophores, stimulate the release of ACh from slices of rat brain (10) and increase the amplitude of end-plate potentials at frog neuromuscular junctions (11), and these actions depend mainly on the external concentration of Ca\(^{2+}\). Thus, it may be fruitful to study the influence of the external concentration of Ca\(^{2+}\) on the modulation by PGs of transmitter release in myenteric neurons.

The effects of indomethacin (IND) and PGs on the response of electrically stimulated guinea pig ileum are contradictory. IND inhibits the contractile response of the ileum to indirect electrical stimulation (EFS), and this inhibition is restored by the addition of PGE\(_2\) (12), or IND does not produce any change in cholinergic responses to transmural stimulation (2).

In this study, the effects of IND and PGE\(_2\) on the spontaneous release and the evoked
releases of ACh were investigated at various external concentrations of Ca²⁺.

Materials and Methods

Preparation of longitudinal muscle strips: Male guinea pigs, weighing 300 to 700 g, were killed by a blow on the head and bled. A length of small intestine was removed, about 10 cm of terminal ileum being discarded. Longitudinal muscle strips, prepared as previously described (1), were tied with cotton threads and mounted in an organ bath that contained 3 ml of Tyrode’s solution of the following composition: 136.9 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.05 mM MgCl₂, 11.9 mM NaHCO₃, 0.4 mM NaH₂PO₄ and 5.6 mM glucose. Physostigmine salicylate (5 µM) and choline chloride (1 µM) were added to the bathing medium. The bathing medium was kept at 37°C and bubbled with 95% O₂ and 5% CO₂.

Release of ACh induced by various stimuli: Before samples of bathing medium for the estimation of the release of ACh were obtained, the strips were equilibrated for 15 min, by perfusing with the Tyrode’s solution at a rate of 1–2 ml/min. Thereafter, the perfusion was stopped and the samples were collected by removing 3 ml of bathing medium and replacing it with 3 ml of fresh Tyrode’s solution at intervals of 1 min. After two samples for measurement of the spontaneous release of ACh were collected, the strips were stimulated by nicotine (6.16 µM), by EFS or by high K⁺ (40 mM). Each stimulus was applied twice with an interval of 19 min between stimuli. The first stimulation was carried out in the absence and the second stimulation was carried out in the presence of the drugs. In experiments to study the effects of [Ca²⁺]₀, the medium was exchanged for Tyrode’s solution that contained various concentrations of Ca²⁺, ranging from 0.45 to 3.6 mM, 17 min before the second stimulation. For the release of ACh induced by EFS at 10 Hz, the stimulation was performed in trains of 20 sec, and bathing fluid was collected for a further 10 sec after cessation of stimuli. EFS was carried out with a pair of Pt electrodes; one electrode was fixed at the top and the other one at the bottom of the strip. The parameters of EFS were: supramaximal voltage (100 V), 0.5 msec duration, total of 200 pulses at 1 or 10 Hz. The high K⁺ (40 mM) Tyrode’s solution was kept iso-osmotic by appropriate changes in the concentration of Na⁺.

All the samples collected were kept on ice until the assay (within 3 hr). At the end of the experiment, the strips were blotted and weighed. The amount of release of ACh resulting from each stimulation was calculated by subtraction of the output during the immediately preceding resting period of 1 min from the total output during the period of stimulation. All results, in both the figures and the text, are given as relative amounts of ACh released, defined in the following way: release of ACh during the second stimulation was expressed as a percentage of that during the first stimulation. In order to evaluate the effects of IND and PGE₂ on the release of ACh at various concentrations of Ca²⁺, the release of ACh in the presence of IND and/or PGE₂ at each concentration of Ca²⁺ was expressed as a percentage of that at the corresponding concentration of Ca²⁺ in the absence of drugs. The spontaneous release of ACh immediately prior to the second stimulation was expressed as a percentage of that immediately prior to the first stimulation.

Assay of released ACh: The collected samples were gently shaken with one-tenth volume of Amberlite XAD-2 for 15 min and filtered to remove endogenous PGs which were presumed to be present in the samples and the added nicotine (1). The aliquots were then assayed for ACh on an isolated longitudinal muscle strip obtained from the most distal region of the guinea pig ileum. Physostigmine salicylate (50 nM) and morphine hydrochloride (10 µM) were added to the Tyrode’s solution in the assay bath in order to increase the sensitivity of the longitudinal strips to ACh and to inhibit endogenous release of ACh, respectively. The medium in the assay bath was kept at 37°C and bubbled with 95% O₂ and 5% CO₂. To eliminate the possible interference in the assay by the drugs present in the collected samples, the responses to the bathing fluids were compared with those of the standard solution of ACh to which the tested drugs were added, at the same final concentration.
The active substance in the collected samples was identified as ACh by demonstrating that it was destroyed by boiling in alkali and by showing that its action was antagonized by atropine and potentiated by physostigmine.

**Drugs:** The drugs used were: acetylcholine chloride (Sigma), indomethacin (Sigma), choline chloride (Wako), physostigmine salicylate (Sigma), morphine hydrochloride (Takeda Chemical Industries) and nicotine (Nakarai). Prostaglandin E₂ was a gift from the Ono Pharmaceutical Company.

All other drugs were of analytical grade. The stock solutions of indomethacin and prostaglandin E₂ were prepared in ethanol. Ethanol at the concentration used did not affect the release of ACh.

The results were analyzed by Student's t-test.

**Results**

In 1.8 mM [Ca²⁺]₀, the amounts of ACh release induced by each kind of stimuli were as follows (nmol/g tissue/min): 575.7±49.5 (spontaneous, n=10), 857.6±99.8 (nicotine, n=10), 503.1±111.8 (EFS at 1 Hz, n=3), 1032.0±103.8 (EFS at 10 Hz, n=3) and 577.7±50.0 (high K⁺, n=3).

**Spontaneous release of ACh:** The increase in [Ca²⁺]₀ from 0.45 to 3.6 mM produced a significant decrease in the spontaneous release of ACh. The values for the ratio (R₂/R₁ × 100) were 186.3±15.7, 139.8±2.7, 96.1±2.3 and 85.7±6.4% for [Ca²⁺]₀ values of 0.45, 0.9, 1.8 and 3.6 mM, respectively (Fig. 1). IND (2.8 μM) inhibited the spontaneous release of ACh between 0.45 and 1.8 mM [Ca²⁺]₀. At 3.6 mM [Ca²⁺]₀, the spontaneous release of ACh was not inhibited by IND (Fig. 2). PGE₂ (14.3 nM) reversed the inhibitory effects of IND on the spontaneous release of ACh (Fig. 2).

**Nicotine-induced release of ACh:** The release of ACh evoked by nicotine (6.16 μM) was augmented significantly at low [Ca²⁺]₀ and then declined progressively as [Ca²⁺]₀ was further raised, as shown in Fig. 1. The values for the ratio (S₂/S₁ × 100) were 177.3±23.9, 153.1±25.4, 111.2±8.9 and 52.4±6.6 for [Ca²⁺]₀ values of 0.45, 0.9, 1.8 and 3.6 mM, respectively. IND (2.8 μM) inhibited the

![Fig. 1](image1.png)

**Fig. 1.** Effects of [Ca²⁺]₀ on the spontaneous (●) and nicotine (●)-, EFS- at 1 Hz (△) or 10 Hz (▲) and high K⁺ (□)-induced release of ACh from guinea pig ileum myenteric plexus. The first stimulation was carried out at 1.8 mM [Ca²⁺]₀ (S₁) and the second one at various [Ca²⁺]₀ (S₂). Symbols and vertical bars represent the mean±S.E. (n=3–10).

![Fig. 2](image2.png)

**Fig. 2.** Influence of [Ca²⁺]₀ on the inhibitory effect of indomethacin (○) and the reversal of the inhibition by prostaglandin E₂ (●) on the spontaneous release of ACh from guinea pig ileum myenteric plexus. Symbols and vertical bars represent the means±S.E. (n=3–10). The release of ACh in the presence of IND and/or PGE₂ at each concentration of Ca²⁺ is expressed as the percentage of that at the corresponding concentration of Ca²⁺ in the absence of drugs. IND (2.8 μM) and PGE₂ (14.3 nM) were added to the medium 17 min and 2 min before the second stimulation (see text), respectively. *P<0.05, **P<0.01, as compared with the corresponding control (no drug). *P<0.05, **P<0.01, as compared with the corresponding IND-treated preparation.
nicotine-induced release of ACh at all tested concentrations of Ca\(^{2+}\) (Fig. 3). At 1.8 mM [Ca\(^{2+}\)]\(_o\), IND inhibited the nicotine-induced release of ACh to about 35% of the control values, and the extent of the inhibition was almost the same at lower concentrations of Ca\(^{2+}\). At 3.6 mM [Ca\(^{2+}\)]\(_o\), the inhibitory effect of IND appeared to be less potent. PGE\(_2\) (14.3 nM) reversed to a considerable extent the inhibitory effect of IND on the nicotine-induced release of ACh at 0.9 to 3.6 mM [Ca\(^{2+}\)]\(_o\) (Fig. 3). The nicotine-induced release of ACh at 3.6 mM [Ca\(^{2+}\)]\(_o\) was restored by PGE\(_2\) to the control level. The restoration caused by PGE\(_2\) decreased slightly at Ca\(^{2+}\) concentrations of 0.9 and 1.8 mM. At 0.45 mM [Ca\(^{2+}\)]\(_o\), PGE\(_2\) produced only a slight restoration (Fig. 3).

**EFS-induced release of ACh**: Unlike in the case of the nicotine-induced release of ACh, the EFS-induced (1 or 10 Hz, 0.5 msec, and 100 V) release of ACh increased in proportion to [Ca\(^{2+}\)]\(_o\). At 0.45 mM [Ca\(^{2+}\)]\(_o\), the release of ACh at 1 Hz was abolished, and the release at 10 Hz decreased to 34.4±7.2% of the control (Fig. 1). The relative amounts of ACh released at 1.8 mM were 122.7±13.8% and 110.9±4.9% of the control for 1 and 10 Hz, respectively (Fig. 1). At 3.6 mM [Ca\(^{2+}\)]\(_o\), the EFS-induced release of ACh increased to 202.0±31.0% and 143.4±13.7% of the control at 1 and 10 Hz, respectively (Fig. 1). IND (2.8 μM) did not modify the EFS-induced release of ACh at any given [Ca\(^{2+}\)]\(_o\) (Fig. 4). At 0.45 mM [Ca\(^{2+}\)]\(_o\), the EFS-induced release of ACh was increased by IND, but this did not reach statistical significance. The addition of PGE\(_2\) (14.3 nM) in the presence of IND had no effect on the EFS-induced release of ACh (Fig. 4).

**Release of ACh induced by high K\(^+\)**: At [Ca\(^{2+}\)]\(_o\) ranging between 0.45 and 3.6 mM, the release of ACh induced by high K\(^+\) increased with the increases in [Ca\(^{2+}\)]\(_o\) in a similar manner to the EFS-induced release of ACh (Fig. 1). IND (2.8 μM) did not modify the high K\(^+\)-induced release of ACh at any given [Ca\(^{2+}\)]\(_o\) (Fig. 5).

**Discussion**

In the present experiments, PGE\(_2\) reversed the inhibitory effect of IND on the nicotine-induced release of ACh at [Ca\(^{2+}\)]\(_o\) between...
IND decreases PG synthesis by inhibiting cyclooxygenase (13). The dose of IND used in this study is expected to inhibit most, if not all, of the PG synthesis in the tissue (14). In addition to inhibiting PG synthesis, IND has various actions (15), but these effects occur at concentrations more than 10 times higher than that used in the present experiments. Thus, the inhibitory effects of IND on the release of ACh can be attributed to its ability to inhibit endogenous PG synthesis. At high \([Ca^{2+}]_o\), the extent of the restoration of activity by PGE2 was slightly greater than that at low \([Ca^{2+}]_o\). These results indicate that the external Ca\(^{2+}\) may be somehow involved in the modulatory effect of PGs on the nicotine-induced release of ACh. PGs have actions as Ca ionophores (16) or activate Ca channels (4). These effects of PGs on the entry of Ca\(^{2+}\) from the external medium may explain the Ca\(^{2+}\)-dependent modulation of the ACh release by PGs. However, in any given \([Ca^{2+}]_o\) tested, IND and PGE2 did not change the amount of ACh released in response to EFS and to high K\(^+\); both those effects depended on \([Ca^{2+}]_o\) (17) and could be depressed by cadmium (18). Therefore, modulation by PGs of the nicotine-induced release of ACh may not be due to activation of Ca channels.

The reason why PGE\(_2\), in the presence of IND, was less effective in the case of the nicotine-induced release of ACh at 0.45 mM \([Ca^{2+}]_o\) is unclear at present. Carafoli and Crovetti (19) showed that the binding of PGE\(_2\) to mitochondrial membranes of rat liver increases with an increase in \([Ca^{2+}]_o\) and that the binding is maximal at 500 \(\mu M\) \([Ca^{2+}]_o\). If this is the case in the guinea pig enteric neurons, the failure of the effect of PGE\(_2\) may be due to the decrease in the binding of PGE\(_2\) to the membranes of cholinergic neurons as a result of the lowering of the external Ca\(^{2+}\) concentration.

PGs raise the level of cyclic adenosine monophosphate (cyclic AMP) in peripheral nerves (20). The increase in the intracellular level of cyclic AMP plays an important role in the excitability of the enteric nerves (21). These evidences suggest the possibility that the main effects of the Ca\(^{2+}\)-independent actions of PGs on the regulation of the nicotine-induced release of ACh involve the adenylate cyclase system.

At 3.6 mM \([Ca^{2+}]_o\), IND did not modify the spontaneous release of ACh, but inhibited the nicotine-induced release of ACh. The release of PGs depends on \([Ca^{2+}]_o\) (22, 23). In the present study, the inhibitory effects of IND on the nicotine-induced and the spontaneous release of ACh were inversely related to \([Ca^{2+}]_o\). These results suggest that the inhibitory effects of IND on the release of PGs may be less potent at high \([Ca^{2+}]_o\) than at low \([Ca^{2+}]_o\). At 3.6 mM \([Ca^{2+}]_o\), therefore, a considerable amount of PGs may be released into the medium, even in the presence of 2.8 \(\mu M\) IND which efficiently prevents the production of PGs. This level of PGs may be sufficient to maintain the spontaneous release of ACh, while the sensitivity of enteric neurons to nicotine may be lowered by the slight decrease of the production of PGs. This suggestion is consistent with our previous conclusions (24) that PGs contribute to the modulation of the nicotine-induced release of ACh more effectively than to the modulation of the spontaneous release of ACh.

IND had no effect on the EFS- and high K\(^+\)-induced release of ACh at any given \([Ca^{2+}]_o\). This result is in agreement with previous reports (1, 14) that PGs may not play an important role in the depolarization of
nerve terminals and axons of enteric neurons, but the result contradicts the report of Ehrenpreis et al. (12) that the contraction of guinea pig ileum induced by stimulation of nerves at 0.1 Hz is inhibited by IND. In this latter study, however, the preparation was treated with IND at a concentration that was 40-fold higher than the concentration used in the present experiments. High concentrations of IND are known to inhibit Ca$^{2+}$ uptake (25) and phosphodiesterase activity (26). Thus, it is highly probable that IND acts directly on smooth muscle to depress its contractile response. An alternative possibility is that the inhibitory effect of IND on the release of ACh depends on the frequency of stimulation. We used EFS of 1 and 10 Hz to induce the release of ACh, while Ehrenpreis and colleagues (12) employed EFS of 0.1 Hz. Kadlec et al. (27) showed that IND significantly inhibits the release of ACh induced by EFS at 0.1 Hz, but not at 5 Hz. The amount of PGs released in response to EFS is frequency-dependent (21), and therefore the amount of PGs released by high frequency may be sufficient to regulate the release of ACh in spite of the presence of IND.

In conclusion, the present results suggest that in guinea pig ileum, external Ca$^{2+}$ may be not essential for the maintenance of the excitability of the enteric neurons by PGs and suggest that the release of ACh induced by nicotine may be more susceptible to modulation by PGs than is the spontaneous release of ACh.

Acknowledgments: We thank the Ono Pharmaceutical Company for gifts of prostaglandin E$_2$. This research was supported by a Grant-in-Aid for Scientific Research (No. 57560288) from the Ministry of Education, Science and Culture of Japan.

References

1 Yagasaki, O., Funaki, H. and Yanagiya, I.: Contribution of endogenous prostaglandins to excitation of the myenteric plexus of guinea pig ileum: are adrenergic factors involved? Eur. J. Pharmacol. 103, 1–8 (1984)
2 Sokunbi, Y.O.O.: Does a prostaglandin modulate cholinergic transmission in the guinea pig ileum. Br. J. Pharmacol. 67, 464P (1979)
3 Ishizawa, K. and Miyazaki, E.: Prostaglandin F$_2$ (PGF$_2$)-induced contraction and calcium movement in longitudinal muscle of guinea pig isolated stomach. Prostaglandins 16, 591–597 (1978)
4 Koyama, Y., Kitayama, S., Dohi, T. and Tsujimoto, A.: Evidence that prostaglandins activate calcium channels to enhance basal and stimulation-evoked catecholamine release from bovine adrenal chromaffin cells in culture. Biochem. Pharmacol. 37, 1725–1730 (1988)
5 Boubler, E., Bukhave, K. and Rask-Madsen, J.: Significance of calcium for the prostaglandin E$_2$-mediated secretory response to 5-hydroxytryptamine in the small intestine of the rat in vivo. Gastroenterology 90, 1972–1977 (1986)
6 Ishizawa, M. and Miyazaki, E.: Calcium and the contractile response to prostaglandins in smooth muscle of guinea pig stomach. Experientia 33, 376–377 (1977)
7 Manstrom, K. and Carafoli, E.: Effects of prostaglandins on the interaction of Ca$^{2+}$ with mitochondria. Arch. Biochem. Biophys. 171, 418–423 (1975)
8 Macnamara, P.B., Roulet, M.J., Gruetter, C.A., Hyman, A.L. and Kadowitz, P.J.: Correlation of prostaglandin-induced mitochondrial calcium release with contraction in bovine intrapulmonary vein. Prostaglandins 20, 311–320 (1980)
9 Gershon, M.: The enteric nervous system. Annu. Rev. Neurosci. 4, 227–272 (1981)
10 Casamenti, F., Mantovani, P. and Pepeu, G.: Stimulation of acetylcholine output from brain slices caused by the ionophores BrX-537A and A23187. Br. J. Pharmacol. 63, 259–265 (1974)
11 Kita, H. and Van der Kloot, W.: Effects of the ionophore X-537A on the acetylcholine release at the frog neuromuscular junction. J. Physiol. (Lond.) 259, 177–198 (1976)
12 Ehrenpreis, S., Greenberg, J. and Belman, S.: Prostaglandins reverse inhibition of electrically-induced contractions of guinea pig ileum by morphine, indomethacin and acetylsalicylic acid. Nature New Biol. 245, 280–282 (1973)
13 Vane, J.R.: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol. 231, 232–235 (1971)
14 Botting, J.H. and Salzmann, R.: The effect of indomethacin on the release of prostaglandin E and acetylsalicylic acid from guinea pig isolated ileum at rest and during field stimulation. Br. J. Pharmacol. 50, 119–124 (1974)
15 Northover, B.J.: Indomethacin—a calcium antagonist. Gen. Pharmacol. 8, 293–296 (1977)
16 Deleers, M., Grognet, P. and Brasseur, R.: Structural considerations for calcium ionophores by prostaglandins. Biochem. Pharmacol. 34, 3831–3836 (1985)
17 Yokoyama, K., Shimizu, M. and Yagasaki, O.
Effect of external $\text{Ca}^{2+}$ on the spontaneous and the various stimuli-induced acetylcholine release from guinea pig ileum myenteric plexus. Japan. J. Pharmacol. 40, 194–197 (1986)

18 Alberts, P., Ogren, V. and Sellstrom, A.: Cadmium inhibition of $[^3\text{H}]$acetylcholine secretion in guinea pig ileum myenteric plexus. Acta Physiol. Scand. 124, 313–316 (1985)

19 Carafoli, E. and Crovetti, F.: Interaction between prostaglandin $E_1$ and calcium at the level of the mitochondrial membrane. Arch. Biochem. Biophys. 154, 40–46 (1973)

20 Kalix, P.: Prostaglandin $E_1$ raises the cAMP content of peripheral nerve tissue. Neurosci. Lett. 12, 361–364 (1979)

21 Zafirov, D.H., Palmer, J.M., Nemeth, P.R. and Wood, J.D.: Cyclic 3',5'-adenosine monophosphate mimics slow synaptic excitation in myenteric plexus. Brain Res. 347, 368–371 (1985)

22 Bergstrom, S., Carlson, L.A. and Weeks, J.R.: The prostaglandins: A family of biologically active lipids. Pharmacol. Rev. 20, 1–48 (1968)

23 Gerritsen, M.E., Nganele, D.M. and Rodrigues, A.M.: Calcium ionophore (A23187)- and arachidonic acid-stimulated prostaglandin release from microvascular endothelial cells: Effects of calcium antagonists and calmodulin inhibitors. J. Pharmacol. Exp. Ther. 240, 837–846 (1987)

24 Takeuchi, T. and Yagasaki, O.: Modulation of acetylcholine release from guinea pig ileum myenteric plexus by arachidonic acid cascade inhibitors. Japan. J. Pharmacol. 45, 434–437 (1987)

25 Northover, B.J.: The effect of indomethacin on calcium, sodium, potassium and magnesium flux in various tissues of the guinea pig. Br. J. Pharmacol. 45, 651–659 (1972)

26 Flores, A.G.A. and Sharp, G.W.G.: Endogenous prostaglandins and osmotic water flow in the toad bladder. Am. J. Physiol. 223, 1392–1397 (1972)

27 Kadlec, O., Masek, K. and Seferna, I.: Modulation by prostaglandins of the release of acetylcholine and noradrenaline in the guinea pig isolated ileum. J. Pharmacol. Exp. Ther. 205, 636–645 (1978)