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Modulating effects of opioids, purine compounds, 5-hydroxytryptamine and prostaglandin E_2 on cholinergic neurotransmission in a guinea-pig oesophagus preparation

YUICHIRO KAMIKAWA* AND YASUO SHIMO

Department of Pharmacology, Dokkyo University School of Medicine, Mibu, Tochigi 321-02, Japan

The submucous plexus-longitudinal muscularis mucosae preparation of the guinea-pig oesophagus was used to study the actions of morphine, opioid peptides, purine compounds, 5-hydroxytryptamine (5-HT) and prostaglandin E_2 (PGE_2) on electrically-induced twitch contractions which are probably mediated by cholinergic nerve stimulation. The twitch contractions were inhibited by morphine (1–100 μM), methionine–enkephalin (1–100 μM) and β-endorphin (0.1–1 μM), but increased by adenosine (1–30 μM), adenosine 5′-triphosphate (1–30 μM), 5-HT (0·01–3 μM) and PGE_2 (1–10 nM). The submaximal contraction induced by acetylcholine (12 or 20 nM) which is nearly equivalent to the twitch contractions was unaffected by morphine, methionine-enkephalin, β-endorphin and 5-HT, but augmented by purine compounds and PGE_2. It is concluded that cholinergic neurotransmission in the submucous plexus-longitudinal muscularis mucosae of the guinea-pig oesophagus is inhibited by morphine and opioid peptides acting at prejunctional opiate receptors, and facilitated by 5-HT, purine compounds and PGE_2 via prejunctional or postjunctional mechanisms.

The motility of the mammalian gastrointestinal tract is regulated not only by extrinsic sympathetic and parasympathetic nerves, but also by the enteric nervous system. The enteric nervous system consists of two intramural plexuses, the myenteric and the submucous plexus (Furness & Costa 1980). The cholinergic neurons in these plexuses act directly on the intestinal smooth muscle and their stimulation produces a contraction via muscarinic receptors. Much evidence showing that endogenous substances such as opioid peptides, purine compounds, 5-hydroxytryptamine (5-HT) and prostaglandins (PGs) can modulate cholinergic neurotransmission via prejunctional and postjunctional mechanisms have been presented using the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum (Ehrenpreis et al 1973; Hughes et al 1975; Vizi & Knoll 1976; Sawynok & Jhamandas 1976; Vizi & Vizi 1978; Gustafsson et al 1980). However, there are few reports on the submucous plexus. The muscularis mucosae of the guinea-pig oesophagus is innervated chiefly by excitatory cholinergic nerves and very sparsely with inhibitory adrenergic nerves (Kamikawa & Shimo 1979). The isolated preparation contains a submucous plexus including cholinergic nerve cell bodies, independent of the myenteric plexus. This led us to examine the modulating effects of endogenous substances on cholinergic neurotransmission in the plexus measured as their effects on the twitch-like contractions of the longitudinal muscularis mucosae induced by electrical stimulation of intramural cholinergic nerves. A brief report has already been made (Kamikawa et al 1981).

* Correspondence.

MATERIALS AND METHODS

Male guinea-pigs (300 to 500 g) were stunned, the oesophagus excised and the isolated muscularis mucosae attached to the submucous plexus was prepared (Kamikawa & Shimo 1979; Kamikawa et al 1982). Briefly, the excised oesophagus was pinned on a cork mat immersed in Tyrode solution. The outer striated muscle coat was cut longitudinally, and gently peeled away leaving an inner tube. The tube including longitudinal muscularis mucosae, about 15 mm long without a load, was immersed in a 15 ml organ bath filled with Tyrode solution of the following composition (mM); NaCl 136·8, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·05, $NaHCO_3$ 11·9, NaH_2PO_4 0·42, disodium edetate 0·03, ascorbic acid 0·12, and glucose 5·56 (pH 7·4). The Tyrode solution always contained 20 μM choline chloride and was bubbled with 5% carbon dioxide in oxygen, and maintained at 37 °C. The preparation was suspended under a 0·3 g load and 60 min was allowed to elapse before experiments were started. Responses of the longitu-

dinal muscularis mucosae were recorded isotonically by means of an isotonic transducer (MEC-1411) and a Nihon Kohden polygraph recorder (RJG-4004).

Electrical stimulation of intramural nerves in the muscularis mucosae was carried out transmurally by means of two coaxial platinum electrodes, the anode in the lumen and the cathode in the organ bath. The stimulation parameters were always 0·1 Hz, 0·5 ms and supramaximal voltage (approx. 40 V). When the strip was electrically stimulated, stable twitch-like contractions were obtained, the height of which was nearly equivalent to that of the contraction caused by exogenously supplied acetylcholine (ACh, 20 nM). The elicited twitch contractions were mediated by stimulation of intramural cholinergic nerves in the muscularis mucosae, since they were completely inhibited by tetrodotoxin (0·2 μM) or atropine (0·2 μM), and augmented by physostigmine (0·1 μM) (Kamikawa & Shimo 1979; Kamikawa et al 1982). The effects of drugs on the twitch contractions were measured as the percentage changes of the original twitch height obtained just before the drug was applied to the bath.

Drugs used were morphine hydrochloride (Dainippon), naloxone hydrochloride (Endo), methionine-enkephalin, β-endorphin (Protein Research Foundation), adenosine, adenosine 5′-triphosphate disodium salts, aminophylline (Sigma), prostaglandin E_2 (Ono), 5-hydroxytryptamine creatinine sulphate (Nakarai), acetylcholine chloride (Daiichi), physostigmine sulphate, atropine sulphate (Wako), tetrodotoxin (Sankyo) and dipyridamole (C. H. Boehringer Sohn). To prepare stock solutions, all drugs were dissolved in 0·9% w/v NaCl solution (saline). Further dilutions were made with Tyrode solution each day. The molar concentrations of drugs described in this paper refer to the final bath concentrations.

RESULTS

Inhibitory effects of morphine and opioid peptides on the electrically-induced twitch contractions

Morphine (1–100 μM) slightly inhibited the twitch contractions of the isolated muscularis mucosae of the guinea-pig oesophagus induced by transmural electrical stimulation. The inhibitory action was varied from preparation to preparation and even at 100 μM the twitches were inhibited only 5–50% (mean about 30%) (Fig. 1A, Fig. 2). Methionine-enkephalin (1–100 μM) also inhibited the twitch contractions, in a concentration-dependent manner, and at 100 μM the twitches were inhibited about 85% (Fig. 1B, Fig. 2). β-Endorphin was most effective in inhibiting the twitches of this preparation and at 1 μM the twitches were inhibited about 84% (Fig. 1C, Fig. 2). The twitch inhibitory actions of morphine and opioid peptides were completely reversed by a narcotic antagonist, naloxone (1 μM, n = 5) (Fig. 1B). This indicates that these opiate agonists may inhibit the twitch response via specific opiate receptors. Since morphine and opioid peptides did not modify the submaximal contraction induced by ACh (12 or 20 nM) (Fig. 1A, C), the opiate receptors responsible for the twitch inhibition might be located on cholinergic nerves in the submucous plexus.

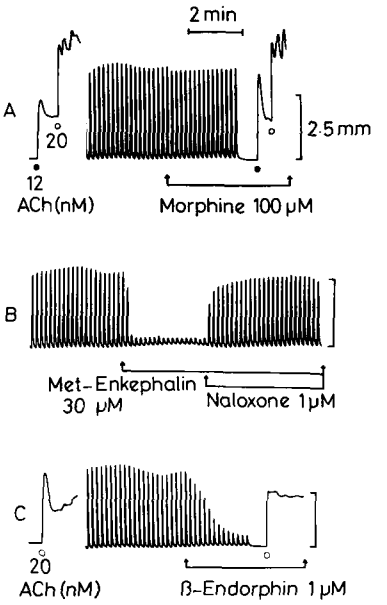

FIG. 1. Inhibitory effects of morphine (100 μM, A), methionine-enkephalin (Met-Enkephalin, 30 μM, B) and β-endorphin (1 μM, C) on the twitch contractions of the isolated muscularis mucosae of the guinea-pig oesophagus induced by electrical stimulation (0·1 Hz, 0·5 ms and supramaximal voltage) and its reversal by naloxone (1 μM, B). The submaximal contractions induced by acetylcholine (ACh, 12 nM at dots and 20 nM at open circle) were unaffected by morphine (A) and β-endorphin (B). Separate preparations were used for morphine, methionine-enkephalin and β-endorphin. Vertical calibrations show 2·5 mm shortening of the tissue.

Facilitatory effects of purine compounds, 5-HT and PGE_2 on the electrically-induced twitch contractions

Adenosine (1–30 μM) increased the electrically-induced twitch contractions of the preparation about 10–20% (Fig. 3). The twitch increasing action was not dependent on the concentration, but was always accompanied with a similar degree of augmentation of the ACh-induced contraction (n = 7). These

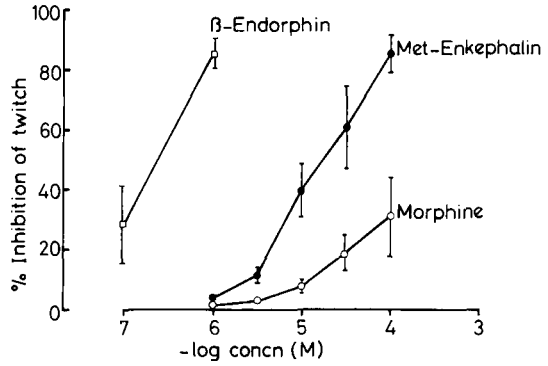

FIG. 2. Concentration-response curves for the twitch inhibitory actions of β-endorphin (□, n = 3), methionine-enkephalin (●, n = 6) and morphine (○, n = 6) in the isolated muscularis mucosae of the guinea-pig oesophagus. Each point represents the mean ± s.e.mean.

effects of adenosine were unaffected by the pretreatment with aminophylline (100 μM, n = 4) and dipyridamole (1 μM, n = 4). Similar but less effective augmentations of the electrically- and ACh-induced contractions were also observed with adenosine 5'-triphosphate (ATP, 1–30 μM) (Fig. 3).

5-HT, at concentrations higher than 0·01 μM, increased the electrically-induced twitch contractions about 5–30% (Fig. 3). The twitch increasing action was not so dependent on the concentration. Above 3 μM, 5-HT produced a contraction of the muscularis mucosae and therefore the action on the twitches could not be quantified accurately. 5-HT did not modify the submaximal contraction induced by ACh (12 or 20 nM) at the concentrations examined (0·01–3 μM) (n = 6).

PGE_2 (1–10 nM) also increased the twitch contractions, in a concentration-dependent manner, and at 10 nM the height of the twitches increased about 50%

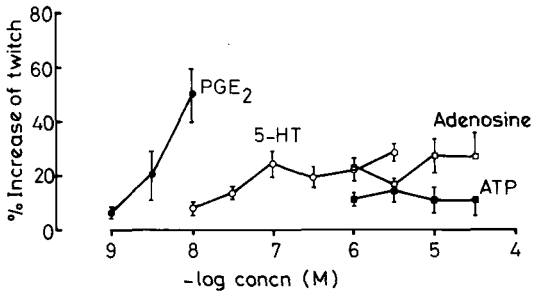

FIG. 3. Concentration-response curves for the twitch increasing actions of PGE_2 (●, n = 5), 5-HT (○, n = 6), adenosine (□, n = 7) and adenosine 5'-triphosphate (ATP, ■, n = 5) in the isolated muscularis mucosae of the guinea-pig oesophagus. Each point represents the mean ± s.e.mean.

(Fig. 3). The submaximal contraction induced by ACh (12 nM) was augmented by PGE_2 to a greater extent than that on the twitches (n = 5). Above 10 nM, PGE_2 produced a contraction of the muscularis mucosae.

DISCUSSION

In the present experiments, the electrically-induced twitch contractions of the submucous plexus-longitudinal muscularis mucosae preparation of the guinea-pig oesophagus were inhibited by morphine and opioid peptides, and increased by purine compounds, 5-HT and PGE_2. The inhibitory actions of morphine and opioid peptides seem to be mediated by the opiate receptors which are located in cholinergic nerves of the submucous plexus and cause a prejunctional reduction of the ACh release, since the inhibitory actions of these substances were completely reversed by naloxone, and were not accompanied with a significant inhibition of the ACh-induced contraction (Fig. 1). The order of potency was β-endorphin, methionine-enkephalin and, much weaker, morphine (Fig. 2). It has been well known that morphine and opioid peptides also inhibit the electrically-induced twitch contractions of the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum via a prejunctional mechanism (Paton 1957; Gyang & Kosterlitz 1966; Hughes et al 1975; Kosterlitz & Hughes 1978; Kamikawa & Shimo 1978). However, β-endorphin, methionine-enkephalin and morphine had similar potencies in the guinea-pig ileum. The differences in the order of potency between two preparations may be due to the different types of opiate receptors, since the presence of multiple types of opiate receptors in various peripheral tissue preparations has been reported (Kosterlitz 1978; Wüster et al 1981). The exact type(s) of opiate receptors in the present tissue is not clearly established from the present results and further studies on the relative potencies of a number of opiate agonists and antagonists in this preparation will be required.

The twitch-increasing action of 5-HT seems to be due to a prejunctional increase of the ACh release from cholinergic nerves in the submucous plexus, since the submaximal contraction to the exogenously supplied ACh was unaffected by 5-HT. On the other hand, twitch-increasing actions of purine compounds and PGE_2 are probably mediated by a postjunctional mechanism which causes sensitization of the muscularis mucosae to the released ACh. In contrast to the present results, it has been demonstrated that purine compounds inhibit the electrically-induced twitch

contractions of the myenteric plexus-longitudinal muscle of the guinea-pig ileum via a prejunctional mechanism (Sawynok & Jhamandas 1976; Vizi & Knoll 1976; Hayashi et al 1978). On the other hand, PGE_2 also had a twitch increasing action on the guinea-pig ileum (Ehrenpreis et al 1973, 1976). Although evidence that the facilitatory action is mediated by a prejunctional increase of the ACh release from the myenteric plexus has been presented (Kadlec et al 1978), Laekeman & Herman (1978) and Gustafsson et al (1980) demonstrated that the action is mediated by a postjunctional mechanism.

It is generally accepted that the enteric nervous system consisting of the myenteric and the submucous plexuses plays an important role in the peristaltic reflex of the gut (Hirst 1979; Holman 1981). There is much supporting evidence that endogenous opioid peptides, purine compounds, 5-HT and PGs might play some modulating roles in the development of peristalsis (Bennett et al 1976; Van Nueten et al 1977; Okwuasaba et al 1977; Costa & Furness 1979). Therefore, endogenous substances may modulate cholinergic neurotransmission by different modes of action between the myenteric and the submucous plexuses.

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REFERENCES

Bennett, A., Eley, K. G., Stockley, H. L. (1976) Br. J. Pharmacol. 57: 335–340

Costa, M., Furness, J. B. (1979) Ibid. 65: 237–248

Ehrenpreis, S., Greenberg, J., Belman, S. (1973) Nature New Biol. 245: 280–282

Ehrenpreis, S., Greenberg, J., Comaty, J. E. (1976) Eur. J. Pharmacol. 39: 331–340

Furness, J. B., Costa, M. (1980) Neuroscience 5: 1–20

Gustafsson, L., Hedqvist, P., Lundgren, G. (1980) Acta Physiol. Scand. 110: 401–411

Gyang, E. A., Kosterlitz, H. W. (1966) Br. J. Pharmacol. 27: 514–527

Hayashi, E., Mori, M., Yamada, S., Kunitomo, M. (1978) Eur. J. Pharmacol. 48: 297–307

Hirst, G. D. S. (1979) Br. Med. Bull. 35: 263–268

Holman, M. E. (1981) in: Bülbring, E., Brading, A. F., Jones, A. N., Tomita, T. (eds) Smooth Muscle: An Assessment of Current Knowledge. Edward Arnold, London, pp 311–338

Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A., Morris, H. R. (1975) Nature 258: 577–579

Kadlec, O., Mašek, K., Šeferna, I. (1978) J. Pharmacol. Exp. Ther. 205: 635–645

Kamikawa, Y., Shimo, Y. (1978) Br. J. Pharmacol. 64: 511–518

Kamikawa, Y., Shimo, Y. (1979) Arch. Int. Pharmacodyn. 238: 220–232

Kamikawa, Y., Shimo, Y., Uchida, K. (1982) Br. J. Pharmacol. 76: 271–277

Kamikawa, Y., Uchida, K., Shimo, Y. (1981) in: 8th Int. Congr. Pharmacol., Tokyo, Abstr. pp 697

Kosterlitz, H. W. (1978) in: Gráf, L., Palkovits, M., Rónai, A. Z. (eds) Endorphins '78, Excerpta Medica, Amsterdam, pp 205–216

Kosterlitz, H. W., Hughes, J. (1978) in: Adler, M. L., Manara, L., Samanin, R. (eds) Factors Affecting the Action of Narcotics. Raven Press, New York, pp 19–37

Laekeman, G. M., Herman, A. G. (1978) Prostaglandins 15: 829–837

Okwuasaba, F. K., Hamilton, J. T., Cook, M. A. (1977) Eur. J. Pharmacol. 43: 181–194

Paton, W. D. M. (1957) Br. J. Pharmacol. 12: 119–127

Sawynok, J., Jhamandas, K. H. (1976) J. Pharmacol. Exp. Ther. 197: 379–390

Van Nueten, J. M., Van Ree, J. M., Vanhoutte, P. M. (1977) Eur. J. Pharmacol. 41: 341–342

Vizi, E. S., Knoll, J. (1976) Neuroscience 1: 391–398

Vizi, V. A., Vizi, E. S. (1978) J. Neural Transm. 42: 127–138

Wüster, M., Schulz, R., Herz, A. (1981) Biochem. Pharmacol. 30: 1883–1887