EFFECTS OF ACETYLCHOLINE RELEASING DRUGS ON ELECTRICAL ACTIVITIES OBTAINED FROM AUERBACH'S PLEXUS IN THE GUINEA PIG ILEUM

Takao SATO, Issei TAKAYANAGI and Keijiro TAKAGI

*Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences,
University of Tokyo, Bunkyo-ku, Tokyo, Japan.*

Accepted November 19, 1973

Abstract—Effects of 5-hydroxytryptamine, sodium picrate, caerulein and pentagastrin on the electrical activities obtained from Auerbach's plexus in the guinea pig ileum were investigated. The drugs used increased the spike frequency and increase of the spike frequency induced was inhibited by morphine but not by atropine and hexamethonium. The muscle spikes obtained simultaneously were inhibited by atropine. These facts indicate that the drugs used stimulate Auerbach's plexus and accelerate the acetylcholine release from the cholinergic post-ganglionic nerve endings. The sites of action of morphine are also discussed.

The autonomic control of gastrointestinal muscle movement is cholinergic excitatory and adrenergic and non-adrenergic inhibitory, however, the process by which movement of the longitudinal muscle is modulated by neural elements is not well known. Electrical activities in Auerbach's plexus have been recently studied (1-8) and in a previous paper (9) these authors classified spikes from Auerbach's plexus in the guinea pig ileum as being of two typical patterns; one is termed a single spike unit which is modified by many drugs affecting the cholinergic transmission and the other is a burst unit which is hardly affected by most pharmacological agents. For these reasons the single spike unit is considered to be concerned with cholinergic transmission (9). The effects of certain acetylcholine releasing drugs on the single spike unit from Auerbach's plexus in the guinea pig ileum are reported herein. Part of these results has already been presented to the Japanese Pharmacological Society (10).

MATERIALS AND METHODS

After sacrificing male guinea pigs (350 to 600 g in wt.) by a blow on the neck, the longitudinal muscle with Auerbach's plexus was carefully removed from a segment of the ileum and was slipped onto a rectangular Lucite holder. The holder was mounted in oxygenated Locke Ringer's solution at $34 \pm 0.5^\circ\text{C}$ in a 10 ml organ bath. A glass-suction electrode, having tip diameters of 30 to 100 μ and resistance of less than about 100 kilohms, was set up so that it directly touched Auerbach's plexus to record electrical activities according to the methods described in our previous report (9).

In order to record mechanical responses to drugs, the longitudinal muscle with Auerbach's plexus from the guinea pig ileum was suspended in a 10 ml organ bath filled

with Locke Ringer's solution kept at $34 \pm 0.5^\circ\text{C}$ and gassed with a mixture of 95% O_2 and 5% CO_2 . Responses to drugs were recorded through an isotonic lever. Locke Ringer's solution used had the following composition: 154 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl_2 , 2.1 mM MgCl_2 , 5.9 mM NaHCO_3 and 2.8 mM glucose. In most cases the results represent the mean of at least 5 experiments.

Drugs used

Agonists: 5-hydroxytryptamine creatinine sulfate, sodium picrate, caerulein and pentagastrin.

Antagonists: atropine sulfate, hexamethonium bromide, nicotine bitartrate and morphine hydrochloride. Drug concentrations are expressed as g/ml of salts in the bath fluid.

RESULTS

5-Hydroxytryptamine increased spike frequency in a concentration of 3×10^{-6} g/ml (Fig. 1) but in some preparations the increase of spike frequency was observed by a lower concentration (3×10^{-7} g/ml) of 5-hydroxytryptamine. The single spike induced by 5-hydroxytryptamine was not affected by a 3 min pretreatment with atropine (10^{-6} g/ml) (Fig. 1), hexamethonium (10^{-5} g/ml) or nicotine (3×10^{-5} g/ml). However Ohkawa and Prosser (4) reported that 5-hydroxytryptamine (up to 10^{-5} g/ml) unaffected the spikes obtained from Auerbach's plexus. Their spikes were similar to our burst unit, which was little affected by drugs (9). Sodium picrate (10^{-4} g/ml) which was already reported to accelerate the acetylcholine liberation from the guinea pig ileum (11, 12), increased the spike frequency (Fig. 2) Caerulein (3×10^{-9} g/ml) (Fig. 3) and pentagastrin (5×10^{-7} g/ml) (Fig. 4) also increased the spike frequency obtained from Auerbach's plexus. Atropine (up to 10^{-6} g/ml) and hexamethonium (10^{-5} g/ml) little affected the spike frequency increased by sodium picrate, caerulein and pentagastrin. Tachyphylaxis was observed by repeated admini-

FIG. 1. Effect of 5-hydroxytryptamine in the absence and presence of atropine.
5-HT : 5-hydroxytryptamine, Atr : atropine, calibrations : 20 μV and 1 sec.

FIG. 2. Effect of sodium picrate in the absence and presence of atropine or hexamethonium.

C₆ : hexamethonium, picrate : sodium picrate, calibrations : 20 μ V and 1 sec.

Note that larger spikes of muscle origin were inhibited by atropine.

stration of a higher concentration (10^{-6} g/ml) of pentagastrin. In some experiments (for example, Fig. 2) with 5-hydroxytryptamine, caerulein, pentagastrin and sodium picrate, large spikes were recorded simultaneously. Since the large spikes were abolished by Mn^{2+} (0.55 mM) but not by tetrodotoxin (10^{-7} g/ml) (9), these were classified as being muscle spikes. Being inhibited by atropine, the muscle spikes were considered to be evoked by release of endogeneous acetylcholine.

In order to examine the antagonistic mechanism between morphine and 5-hydroxytryptamine or caerulein, the relationship between the frequencies of single spikes and the contraction of the longitudinal muscle strip was tested. Morphine (10^{-6} g/ml) reduced the frequency of the single spikes induced by 5-hydroxytryptamine (3×10^{-6} g/ml) to $65 \pm 12.4\%$ (mean $\pm$ S.E., no. of experiments: 10) and 5-hydroxytryptamine-induced contraction of the isolated longitudinal muscle strip to $68.7 \pm 5\%$ (mean $\pm$ S.E., no. of experiments: 10). There was no significant difference between the two data at a 95% probability level. In the experiments with caerulein (3×10^{-6} g/ml) morphine (10^{-6} g/ml) reduced the spike frequency to $26.4 \pm 11.3\%$ (mean $\pm$ S.E., no. of experiments: 16) and the contraction of the longitudinal muscle strip to $10.9 \pm 3.2\%$ (mean $\pm$ S.E., no. of experiments: 10). Though morphine depressed the contraction of the longitudinal muscle evoked by caerulein more

FIG. 3. Effect of caerulein in the absence and presence of atropine or hexamethonium. C_6 : hexamethonium, calibrations: 20 μ V and 1 sec.

FIG. 4. Effect of pentagastrin in the presence of atropine. Atr: atropine, calibrations: 20 μ V and 1 sec.

Note that tachyphylaxis was observed by a repeated administration of pentagastrin (10^{-6} g/ml).

strongly than the spike frequency induced by caerulein, there was no significant difference between the two data at a 95% probability level.

DISCUSSION

Many investigators (11–21) concluded that since contractions of the guinea pig ileum produced by 5-hydroxytryptamine, sodium picrate, caerulein, pentagastrin, tetragastrin and gastrin were inhibited by atropine, tetrodotoxin and morphine but not by hexamethonium, these agonists accelerate the acetylcholine liberation and contract the ileum, and that the sites of action may be different from the nicotinic receptor. In the present experiments, the spike frequency obtained from Auerbach's plexus was increased by the application of agonists used yet the action of the agonists was little affected by atropine and hexamethonium. Therefore, it can be concluded that the sites of action of the agonists used herein are in Auerbach's plexus, and that the agonists modify to a minimum extent the electrical activities of Auerbach's plexus through muscarinic and nicotinic receptors. Furthermore the fact that the muscle spikes, observed in some experiments with the acetylcholine releasing drugs (Fig. 2), were inhibited by atropine suggests that the drugs used accelerate the acetylcholine release and contract the ileum. These conclusions support the previous findings (11–21).

If morphine inhibits the cholinergic transmission at Auerbach's plexus and also at the ending of the postganglionic nerve fibres, the mechanical response of the longitudinal muscle to caerulein will be inhibited more strongly than the electrical activities of Auerbach's plexus induced by caerulein, since caerulein has no direct action on muscle (15, 17) and also that morphine has not the muscle relaxing action in the concentration used herein (22). Morphine depressed the contraction of the longitudinal muscle induced by caerulein more strongly than the spike frequency obtained from Auerbach's plexus, although there is no significant difference between the two data. Morphine is therefore considered to inhibit the cholinergic transmission mainly at Auerbach's plexus and partly at the cholinergic postganglionic nerve endings as previously reported (9).

In the guinea pig ileum, contraction induced by 5-hydroxytryptamine is mediated through two kinds of receptors (19). One, M-receptor, which is present in nervous structures, can be blocked by morphine, tetrodotoxin and atropine. The other, D-receptor, which is in the smooth muscle fibres, can be blocked by dibenzylamine and so-called 5-hydroxytryptamine inhibitors. Brownlee and Johnson (20) reported that contraction of the guinea pig ileum induced by 5-hydroxytryptamine in low concentrations was mainly mediated through M-receptor in the nervous structures, although they also recognized the existence of two kinds of receptors in the guinea pig ileum. In the present study, morphine inhibited the contraction of the isolated longitudinal muscle to 5-hydroxytryptamine as well as the electrical activities induced by 5-hydroxytryptamine. These results suggest that 5-hydroxytryptamine contracts the guinea pig ileum through mainly M-receptor and partly D-receptor in a rather high concentration of 3×10^{-6} g/ml (21).

REFERENCES

- 1) YOKOYAMA, S.: *Pflügers Arch.* **288**, 95 (1966)
- 2) WOOD, J.D.: *Am. J. Physiol.* **219**, 159 (1970)
- 3) OHKAWA, H. AND PROSSER, C.L.: *Am. J. Physiol.* **222**, 1412 (1972)
- 4) OHKAWA, H. AND PROSSER, C.L.: *Am. J. Physiol.* **222**, 1420 (1972)
- 5) WOOD, J.D. AND MAYER, C.J.: *Pflügers Arch.* **338**, 247 (1973)
- 6) HIRST, G.D.S., HOLMAN, M.E., PROSSER, C.L. AND SPENCE, I.: *J. Physiol.* **225**, 60p (1972)
- 7) KOSTERLITZ, H.W. AND LYDON, R.J.: *Br. J. Pharmacol. Chemother.* **43**, 74 (1971)
- 8) NISHI, W. AND NORTH, R.A.: *J. Physiol.* **231**, 471 (1973)
- 9) SATO, T., TAKAYANAGI, I. AND TAKAGI, K.: *Japan. J. Pharmacol.* **23**, 665 (1973)
- 10) SATO, T., TAKAYANAGI, I. AND TAKAGI, K.: *Japan. J. Pharmacol.* **23**, suppl., 21 (1973)
- 11) TAKAGI, K. AND TAKAYANAGI, I.: *Nature* **193**, 589 (1962)
- 12) TAKAGI, K. AND TAKAYANAGI, I.: *Archs. int. Pharmacodyn. Ther.* **155**, 373 (1965)
- 13) BERTACCINI, G., DE CARO, G., ENDEAN, E., ERSPAMER, V. AND IMPICCIATORE, M.: *J. Pharmacol. Chemother.* **34**, 291 (1968)
- 14) FRICO, G.M., LECCHINI, S., FALASCHI, C., DEI TACCA, M. AND CREMA, A.: *Naunyn-Schmidbergs Arch. Pharmacol.* **269**, 44 (1971)
- 15) VIZI, E.S., BERTACCINI, G., IMPICCIATORE, M. AND KNOLL, J.: *European J. Pharmacol.* **17**, 175 (1972)
- 16) VIZI, E.A.: *Br. J. Pharmacol. Chemother.* **47**, 765 (1973)
- 17) NAKAMURA, N., KOYAMA, Y., KOJIMA, T. AND TAKAHIRA, H.: *Japan. J. Pharmacol.* **23**, 107 (1973)
- 18) TACHIBANA, K., KABENO, H. AND KOBAYASHI, Y.: *Japan. J. Pharmacol.* **23**, 233 (1973)
- 19) GADDUM, J.H. AND PICARELLI, Z.P.: *Br. J. Pharmacol. Chemother.* **12**, 323 (1957)
- 20) BROWNLEE, G. AND JOHNSON, E.S.: *Br. J. Pharmacol. Chemother.* **21**, 306 (1963)
- 21) OGURA, Y., MORI, Y. AND WATANABE, Y.: *J. Pharmacol. exp Ther.* **154**, 456 (1964)
- 22) PATON, W.D.M.: *Br. J. Pharmacol. Chemother.* **12**, 119 (1957)