Medical Journal of the
Islamic Republic of Iran

Volume 4
Number 1
Bahar 1369
Ramazan 1410
Spring 1990

ENDOGENOUS RELEASE OF OPIATES BY REPETITIVE ELECTRICAL FIELD STIMULATION IN THE GUINEA-PIG AND RAT ILEAL LONGITUDINAL MUSCLE

A. NAJAFI-FARASHAH, Ph.D.

From the Department of Physiology, Medical School, Yazd University of Medical Sciences, Yazd, Islamic Republic of Iran.

ABSTRACT

The effect of repetitive electrical field stimulation and the response of the guinea-pig and rat ileal longitudinal muscle to single pulse stimulations was examined. Single pulse field stimulation produced twitch contraction which was inhibited by repetitive field stimulation (10 Hz, 40V, 0.5 msec for 5 m). This inhibition was largely, though never completely, reversed by naloxone. Contractions due to exogenous acetylcholine and histamine were also inhibited after repetitive field stimulation. The inhibition of acetylcholine response was party reversed by naloxone whereas that of histamine was not. Contractions due to single pulse field stimulation or to either acetylcholine or histamine were inhibited by prior exposure to high concentrations of acetylcholine as a substitute for high frequency stimulation. The inhibitory responses were resistant to naloxone. The inhibitory responses to acetylcholine and histamine after exposure to the lowest concentration of acetylcholine was seen in preparations treated with tetrodotoxin or hemicholinium. The inhibition of the histamine response by acetylcholine pretreatment was prevented by mepyramine. Response to histamine, but not those to single pulse field stimulation or acetylcholine, were inhibited by prior exposure to histamine. It is concluded that repetitive field stimulation possibly initiates two distinct inhibitory processes. One involves the release of endogenous opiates and is probably mediated by inhibition of acetylcholine release. The second type of inhibition is not mediated by endogenous opiates and can be explained by post-junctional desensitization. The non-specific desensitization to histamine is probably a consequence of histamine release from mast cells by acetylcholine.

MJIRI, Vol.4, No.1, 53-59, 1990

INTRODUCTION

The presence of an opiate ligand has been shown in several parts of the gastrointestinal tract of different species.[1-7]

Morphine and opiate agonists reduce the spontaneous release of acetylcholine in the myenteric plexus as well as electrically-evoked acetylcholine release in the gut and brain.[8-14]

The release of endogenous opiates from the guinea-pig myenteric plexus-longitudinal muscle preparation has been demonstrated by producing a naloxone-sensitive inhibition of the contractions of the longitudinal muscle. This morphine-like inhibition was eli-

cited by repetitive transmural electrical stimulation.[15,16] They observed a marked inhibition of the contractions to single pluse stimulation after a period of stimulation at 10 Hz. This inhibition was largely, though never completely, reversed by naloxone.

Kamikawa and Shimo (1978) showed the antagonistic effect of compound 48/80, a narcotic antagonist like naloxone, on the inhibitory actions of morphine and met-enkephalin on electrically-induced cholinergic contractions of the guinea-pig ileum. Shimo and Ishii (1978) demonstrated that morphine has no inhibitory effect of the adrenergic inhibitory response of the taenia to perivascular nerve stimulation. On the other hand, it depresses the non-adrenergic inhibitory response of the taenia to transmural electrical stimulation via activation of opiate receptors which are probably located in the myenteric plexus of the taenia; therefore, opiate receptors and endogenous opiates are present in the myenteric plexus of the gastrointestinal tract and their best known action is the inhibition of transmitter release, particularly from cholinergic neurons.[18,19] The aim of this study was to investigate further the nature of the inhibitory effect of repetitive electrical field stimulation on the "twitch" responses of the guinea-pig and rat ileal longitudinal muscle. A preliminary account of this work was presented to the Physiological Society.[20]

MATERIALS AND METHODS:

Adult guinea-pigs (200-800 g) and rats (250-350 g) of either sex were killed by stunning and bleeding. The abdomen was opened by a midline incision and segments of either the proximal or middle ileum were quickly removed and placed in a modified Kreb's solution.[21]

Two types of ileal preparations were used. One was an intact segment of 1-3 cm and the other was a myenteric plexus-longitudinal muscle preparation which was prepared by removing the mesentery and opening a 3 cm-long segment of ileum longitudinally along the mesenteric border. A fine scalpel was used to slip off the mucosal and circular muscle layers leaving the myenteric plexus intact.[22] The tissues were set up in 25 ml baths, bubbled with a mixture of 95% O_2 and 5% CO_2 and maintained at 37°C.

The intact ileum segment was attached to holder with a platinum electrode passed through the lumen and the other end attached to a force transducer (Dynamometer, UFI, 125 g). The longitudinal muscle strip was passed through two platinum ring electrodes. The mechanical activity of the longitudinal muscle was recorded isometrically at resting tension of 0.5-1 g.[23]

A Grass 244 stimulator was used to deliver trains of rectangular pulses to the wire electrodes. The signals from the transducer were amplified and recorded on a

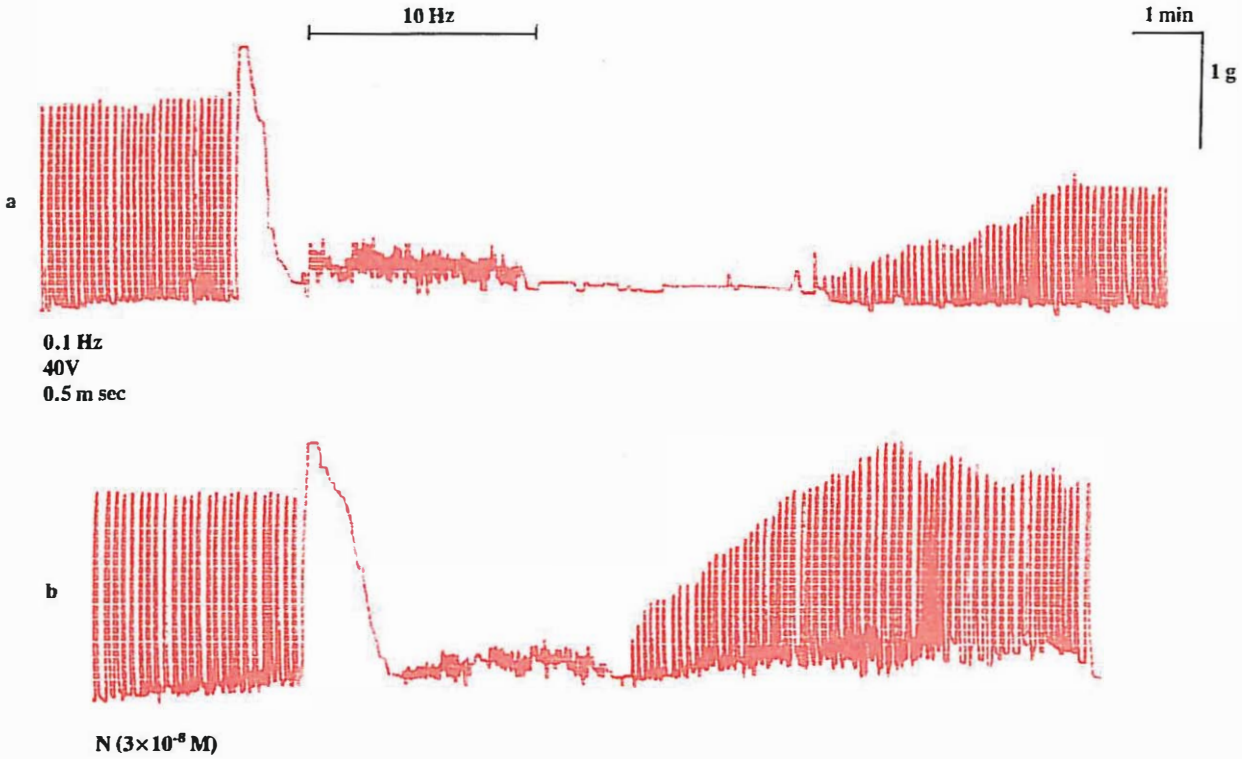

Fig. 1. The inhibitory effect of repetitive electrical field stimulation (10 Hz for 5 min) on the "twitch" responses of the guinea-pig ileal, longitudinal muscle strips (a) and partial reversal by naloxone (N) (b) (n = 18).

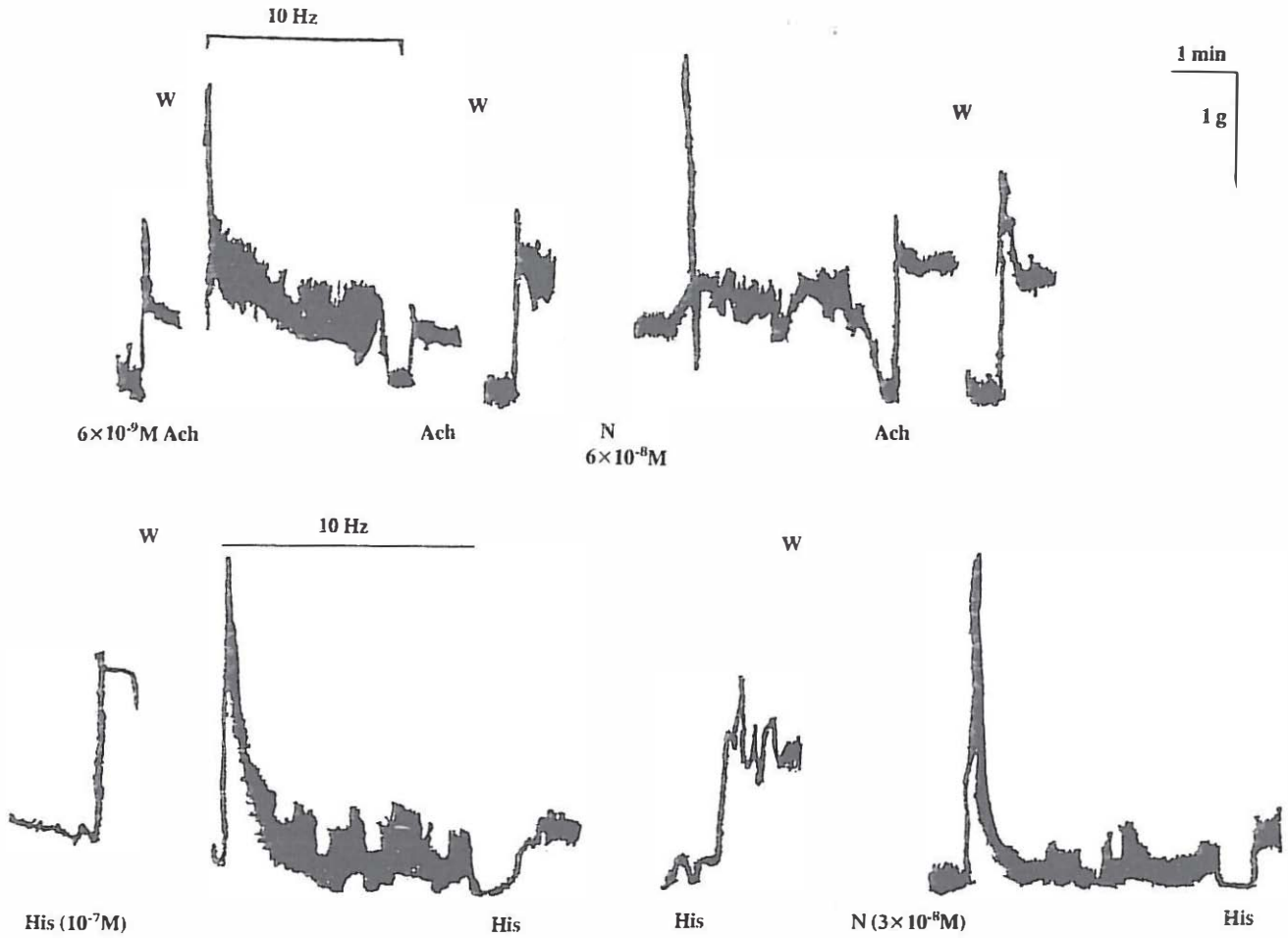

Fig. 2.
Upper traces:
The inhibitory effect of repetitive electrical field stimulation on the response to exogenous acetylcholine (Ach) and partial reversal by naloxone (N) in the isolated guinea-pig ileum (n = 7).
Lower traces:
The inhibitory effect of repetitive electrical field stimulation on the response to exogenous histamine (His) and lack of effect of naloxone in the isolated rat ileum (n=8).

Devices M4 or a Backman R 611 recorder.

The tissue was given at least 30 min to settle. Drugs were added directly to the bath and the concentrations quoted are the final concentrations in the bath.

RESULTS

The twitch contractions in response to single pulse field stimulation (0.1 Hz, 40 V, 0.5 ms) were recorded for 15 min for either intact ileum segments or longitudinal muscle of the myenteric plexus preparation of rat or guinea-pig. When a consistent baseline was obtained a high frequency stimulation of 10 Hz, 40 V, 0.5 ms for 1-5 min was applied, followed by a return to the low frequency again. The twitch contractions in response to single pulse field stimulation were inhibited following repetitive field stimulation. This inhibition was partially reversed by naloxone (10^{-8}-5×10^{-8} M) (Fig. 1).

The peak contraction response to exogenous acetylcholine (10^{-8}M-10^{-9}M), bethanechol (10^{-6}M-10^{-7}M) and histamine (10^{-7}M) was reduced after a long-term high frequency stimulation. Addition of naloxone to the organ bath partially reversed the inhibitory response to acetylcholine or bethanechol, but the inhibition of histamine response was unaffected by naloxone (Fig 2).

The effect of high concentrations of acetylcholine (10^{-5}-10^{-7}M) on twitch contractions or responses to low concentrations of acetylcholine was used as an alternative to long-term repetitive electrical field stimulation to study the post-junctional effects of acetylcholine.

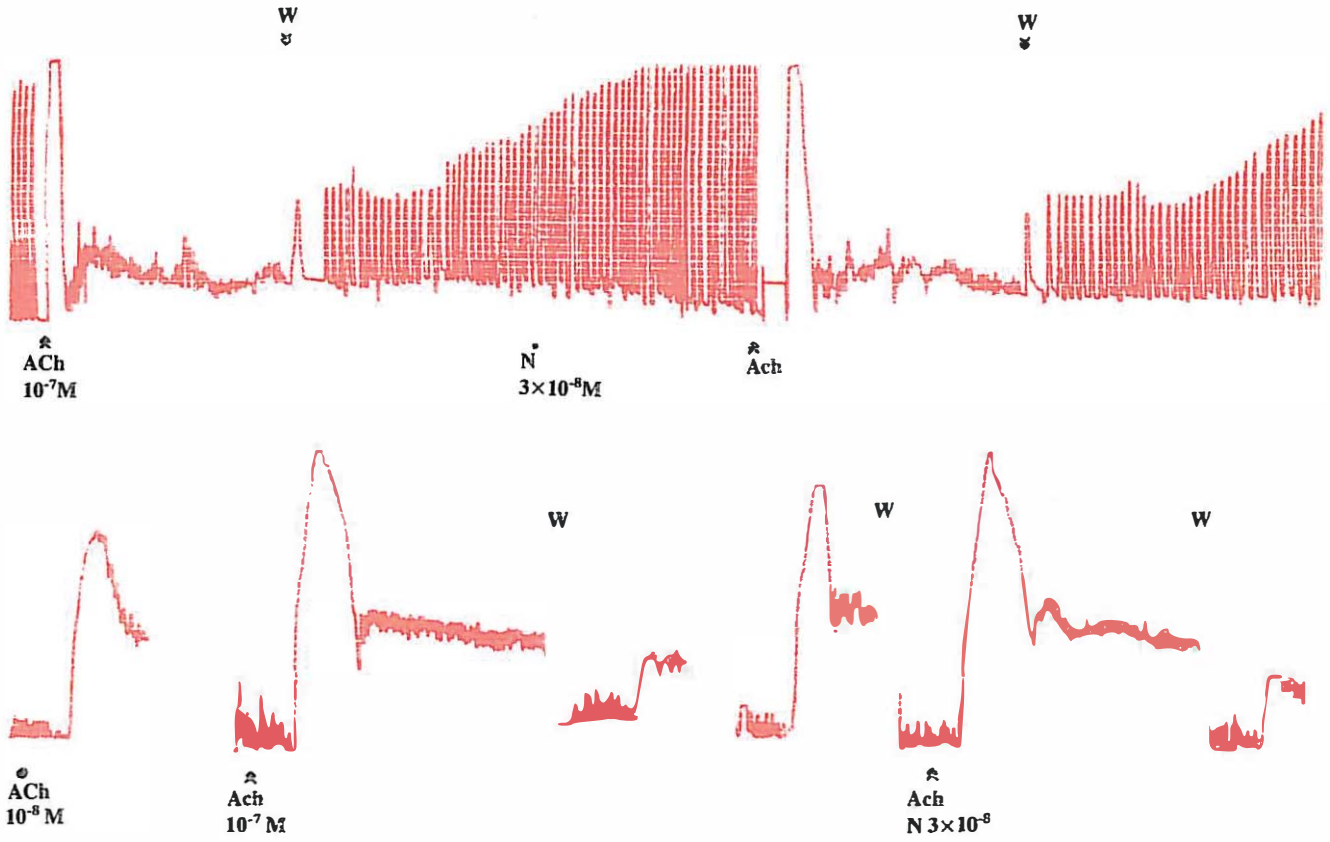

Fig. 3.
Upper-traces:
The inhibitory effectof exogenous acetylcholine (Ach) on the twitch responses of the rat ileal longitudinal muscle strips. Note lack of effect of naloxone (N) on this inhibition (n=6).
Lower-traces:
The inhibitory effect of high concentrations of exogenous acetylcho line (Ach) on the response to low concentrations of Ach. Note lack of effect of naloxone on this inhibition (n = 8).

This part of the experiment was carried out mostly on intact ileum of rats. Naloxone (10^{-7}-10^{-9}M) did not reverse either the inhibitory effect on the twitch contractions or the inhibitory effect of low concentrations of acetylcholine caused by application of high concentrations of exogenous acetylcholine for 30 s-2 min (Fig. 3).

High concentrations of exogenous histamine (5×10^{-6}-5×10^{-5}M) for 2 min did not inhibit twitch responses or response to a moderate concentration of acetylcholine (10^{-9}M), whereas it did inhibit the response to low concentrations (10^{-8}-10^{-7}M) of histamine (Fig. 4).

Addition of either atropine or hyoscine (10^{-6}-10^{-5}M) to the organ bath blocked all responses to the transmural electrical stimulation as well as exogenous acetylcholine, but did not abolish histamine-induced contraction. Tetrodotoxin (10^{-5}-10^{-4}M), which blocked all responses to electrical stimulation, did not alter responses to exogenous acetylcholine or histamine. However, high concentration of acetylcholine (5×10^{-6}-10^{-5}M) applied for 2 min, inhibited the response to single moderate concentrations of acetylcholine (5×10^{-10}-2×10^{-9}M) or histamine (10^{-7}M) and addition of tetrodotoxin did not alter this result. Application of acetylcholine (5×10^{-6}-5×10^{-5}M) for 30 s was found to decrease the response to histamine (2×10^{-7}M) even when followed by washing four times over a period of 2 min. Mepyramine (10^{-8}-10^{-7}M) for 30 s blocked all reponses to exogenous histamine. The effect of mepyramine was partially reversed by washing four times over a period of 2 min, the response to this, however, being still slightly reduced after this period of time ($P<0.01$). Addition of acetylcholine (5×10^{-6}-5×10^{-5}M) caused a marked suppression of subsequent responses to histamine (2×10^{-7}M). The second dose of histamine was given after washing out acetylcholine four times over a period of 2 min. The degree of suppression was significantly greater ($p<0.05$) than reduction seen after washing out mepyramine under

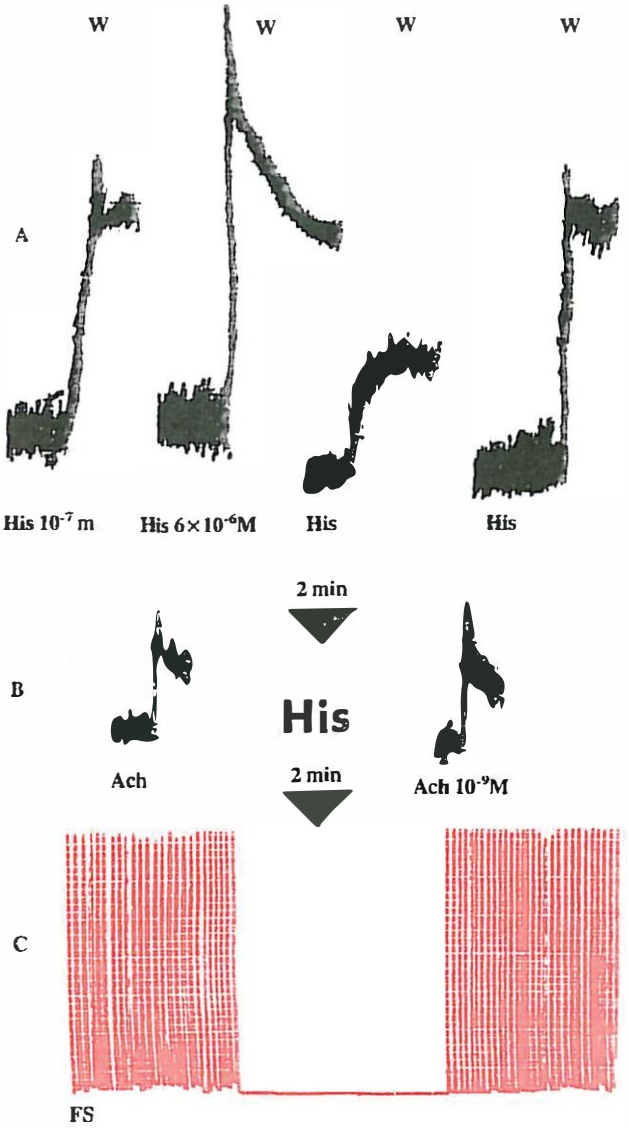

Fig. 4.
A-The inhibitory effect of high concentrations of exogenous histamine (His) on the response to low concentrations of histamine in the isolated guinea-pig and rat ileum (n = 10).
B & C-Lack of effect of high concentrations of exogenous histamine on the acetylcholine (Ach) responses and the twitch responses (Fs) of the guinea-pig and rat ileum (n = 10).

the same conditions. When mepyramine was added to the acetylcholine, the responses to histamine 2 min. later, after washing four times, was reduced. The reduction, however, was not significantly different from that with mepyramine alone (>0.01) but was significantly lower than with acetylcholine alone ($p<0.05$). These results are summarized in Table I. Moreover, the inhibitory effect of high concentrations of histamine on subsequent low concentrations of histamine was prevented by mepyramine (Fig. 5). On the other hand, in the presence of hemicholinium

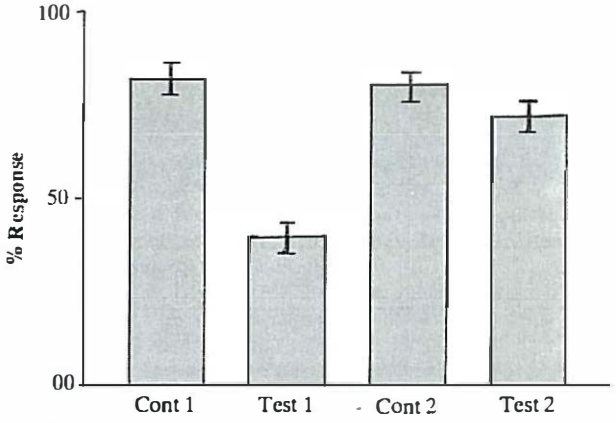

Fig. 5.
The inhibitory effect of high concentrations of exogenous histamine on the response to low concentration of histamine in the isolated guinea-pig ileum and reversal by mepyramine. Control (cont) 1 and 2 represent the mean responses to low concentrations of histamine before application of high concentration of histamine. Test 1 represents the mean responses to low concentrations of histamine after exposure to high concentrations of histamine. Test 2 represents the mean responses to low concentrations of histamine and mepyramine (n = 10). the difference between Test 1 and Test 2 was highly significant ($p<0.001$).

(2×10^{-8}-2×10^{-7}M) twitch contractions in response to single pulse field stimulation after about one hour's recording were almost blocked. Hemicholinium also reduced responses to exogenous acetylcholine in this case, high concentrations of acetylcholine applied for 1 min. inhibited the response to moderate concentration of acetylcholine.

DISCUSSION

The present study confirms and extends earlier findings on the inhibitory effect of repetitive electrical field stimulation (10 Hz) on "twitch" responses of guinea-pig ileum segments.[15,16]

In addition it has been shown in this study that the same inhibitory process is found in the rat ileum.

In agreement with the previous finding of Puig, et al. naloxone only partially reversed the inhibitory effect of high frequency stimulation on twitch responses. Therefore, it suggests that there are two components of this inhibition: one is naloxone-sensitive and the other is naloxone-resistant. Since part of this inhibition is reversed by naloxone (an opiate antagonist)[24] this suggests the involvement of endogenous opiates which when released then probably activate Mu receptors.[25-28]

The twitch responses to low frequency stimulation of the guinea-pig ileum are known to be due to a release of acetylcholine from the myenteric plexus.[9,27,29] Moreover, the inhibition of acetylcholine release by

Table I. The effect of mepyramine, acetylcholine and mepyramine-acetylcholine on histamine responses in the isolated rat ileum.

Drug	Mean reduction in second histamine response	n	p		
Mep(10^{-8}-10^{-7}M)	21%	8	<0.01	p<0.05	p>0.1
Ach(5×10^{-6}-5×10^{-5},)	27.5%	8	< 0.05		
Ach+Mep	21%	8	<0.01	p<0.05	

opiates has been well established.[8,12,16,29-32]

The electrical field stimulation of the isolated ileum-induced contractions were abolished by tetrodotoxin and atropine, demonstrating that this response is induced by excitation of intramural cholinergic neurons.

The inhibitory effect of high concentrations of exogenous acetylcholine on both the twitch contractions and the responses to subsequent low concentrations of acetylcholine was unaffected by naloxone, suggesting a naloxone-resistant process similar to the naloxone-resistant component of the nerve-mediated inhibition. This naloxone-resistant inhibition was obtained also with tetrodotoxin-pretreated preparations and therefore, is non-nervous in origin. The naloxone-resistant component of the nerve-mediated inhibition could be due to activation of different opiate receptors or to postjunctional desensitization. The present study has investigated the possibility that there is a postjunctional desensitization. Evidence which supports this suggestion is that high concentrations of acetylcholine reduced responses to subsequent low concentrations of acetylcholine. This inhibition was naloxone-resistant. Moreover, hemicholinium, which prevents synthesis of acetylcholine[33,34] did not change the inhibitory effect of high concentrations of acetylcholine on responses to subsequent low concentration of acetylcholine.

Both the repetitive transmural electrical stimulation and the exogenous acetylcholine inhibited response to subsequent exogenous histamine which was unaffected by naloxone, suggesting the existence of a non-specific desensitization to histamine. Connection between cholinergic neurons and histamine stores has been established and histamine release has been shown to occur in response to cholinergic agonists or cholinergic nerve stimulation in the submaxillary and parotid gland of cats and dogs, as well as guinea-pig hypothalamus.[35,36] Moreover, acetylcholine-released histamine in the rat mast cell has been blocked by atropine.[37,38] Therefore, it has been concluded that histamine release by acetylcholine is through muscarinic receptors.[39,41]

Since firstly mepyramine prevented the inhibitory effect of exogenous acetylcholine on histamine response, secondly exogenous histamine had no effect on either twitch contractions or responses to subsequent exogenous acetylcholine, and thirdly high concentrations of exogenous histamine inhibited responses to subsequent low concentration of histamine and this inhibitory effect was prevented by mepyramine, it is concluded that the non-specific desensitization to histamine can be satisfactorily explained as postjunctional desensitization caused by histamine release due to acetylcholine. The fact that repetitive field stimulation also caused a naloxone-resistant desensitization to histamine provides very strong support that postjunctional effects are the main cause of the naloxone-resistant reduction in the twitch responses following repetitive field stimulation. Therefore, it is concluded that repetitive field stimulation possibly initiates two distinct inhibitory processes. One involves the release of endogenous opiates and is probably mediated by inhibition of acetylcholine release. The second type of inhibition is not mediated by endogenous opiates and can be explained by postjunctional desensitization following acetylcholine release.

REFERENCES

1. Hughes J, Kosterlitz H W, Smith T W: The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues. Br J Pharmacol 61:639-647, 1977.
2. Nijkamp F P, Van Ree J M: Effects of endorphins on different parts of the gastrointestinal tract of rat and guinea-pig in vitro. Br J Pharmacol 68: 599- 606, 1980.
3. Kromer W, Pretzlaff W, Wolnoff R: Regional distribution of an opioid mechanism in the guinea-pig isolated intestine. J Pharm Phramcol 33: 98-101, 1981.
4. Blanquet F, Bouvier M, Gonella J: Effect of enkephalins and morphine on spontaneous electrical activity and on junction potentials elicited by parasympathetic nerve stimulation in cat and rabbit colon. Br J Pharmacol 77: 419-429, 1982.
5. Horacek J, Kadlec O: An interaction of endogenous and exogenous opiates in the guinea-pig isolated ileum. Arch Int Pharmacodyn 267: 13-22, 1984.
6. Takemori AE, Portoghese PS: Receptors for opioid peptides in the guinea-pig ileum. J Pharmacol Exp Ther 235: 389-392, 1985.
7. Gilbert R J, Sarna SK, Harder DR: Effect of morphine on electrophysiological properties of circular and longitudinal muscles. Am J Physiol 252: 333-8, 1987.
8. Cowie AL, Kosterlitz HW, Waterfield AA: Factors influencing the release of acetylcholine from the myenteric plexus of the ileum of the guinea-pig and rabbit. Br J Pharmcol 64: 565-580, 1978.
9. Down JA, Szerb JD: Kinetics of morphine-sensitive ^{3}H-acetylcholine release from the guinea-pig myenteric plexus. Br J Pharmacol 68: 47-55, 1980.
10. Beani L, Bianchi C, Sinischalchi A: Naloxone reversal of morphine inhibition on electrically-evoked Ach release from guinea-pig thalamus in vitro. Br J Pharmacol 72: 158, 1981.
11. Goldstein DJ, Ropchak TG, Keiser HIR, Atta GJ, Argiolas A, Pisano JJ: Bradykinin reverses the effect of opiates in the gut by

enhancing acetylcholine release. J Biol Chem 258: 12122-12124, 1983.
12. Vizi Y, Ono K, Vizi, VA, Duncalf D, Foldes FF: Presynaptic inhibitory effect of Met-Enkephalin on ^{14}C acetylcholine release from the myenteric plexus and its interaction with muscarinic negative feedback inhibition. J Pharmacol Exp Ther 230: 493-499, 1984.
13. Bardon T, Ruckebusch Y: Comparative effect of opiate agonists on proximal and distal colonic motility in dogs. Eur J Pharmacol 110: 329-334, 1985.
14. Schick K, Schusdziarra V: Physiological-pathophysiological and pharmacological aspects of exogenous and endogenous opiates. Clin Physiol Biochem 3: 43-60, 1985.
15. Puig MM, Gascon P, Craviso GL, Musacchio JM: Endogenous opiate receptor ligand-electrically induced release in the guinea-pig ileum. Science 195: 419-420, 1977.
16. Fosbraey P, Johnson E S: Release-modulating acetylcholine receptors on cholinergic neurones of the guinea-pig ileum. Br J Pharmacol 68: 289-300, 1980.
17. Kamikawa Y, Shimo Y: Antagonistic effect of compound 48/80 on the inhibitory actions of morphine and methionine-enkephalin on electrically induced contractions of the guinea-pig ileum. Br J Pharmacol 64: 511-518, 1978.
18. Shimo Y, Ishii T: Effect of morphine on non-adrenergic inhibitory responses of the guinea-pig taenia coli. J Pharm Pharmacol 30: 596-597, 1978.
19. Tonini M, Onorl L, Perucca E, Manzo L, Ponti F, Crema A: Depression by morphine of the excitability of intrinsic inhibitory neurons in the guinea-pig colon. Eur J Pharmacol 115: 317-320, 1985.
20. Davison J S, Najafi-Farashah A, Pearson GT, Petersen OH: The effect of repetitive electrical field stimulation on the response of guinea-pig ileal longitudinal muscle to single pulse stimulation. J Physiol 310: 58-59, 1980.
21. Davison JS, Najafi-Farashah A: The possible role of cholecystokinin pancreozymin (CCK/PZ) in the peristalic reflex-studies with competitive antagonists to CCK/PZ and related peptides. In: Motility of the Digestive Tract. Edited by M. Wienbeck, Raven Press, New York, pp 79-85, 1982.
22. Wood JD: Electrical activity from single neurons in Auerbach's plexus. Am J Physiol 219: 159-169, 1770.
23. Davison JS, Najafi-farashah A: Dibutyryl cyclic GMP, a competitive inhibitor of cholecystokinin-pancreozymin and related peptides in the gallbladder and ileum. Can J Physiol Pharmacol 59: 1100-1104, 1981.
24. Hughes J, Smith T, Morgan B, Fothergill L: Purification and properties of enkephalin-the possible endogenous ligand for the morphine receptor. Life Sci 16: 1753-1758, 1975.
25. Huidobro-Toro JP, Way EL: Comparative study on the effect of morphine and the opioid-like peptides in the vas deferens of rodents: species and strain differences, evidence for multiple opiate receptors. Life Sci 28: 1331-1336, 1981.
26. Clson GA, Olson RD, Kastin AJ, Coy DH: Endogenousopiates: review. Peptides 2: 349-369, 1981.
27. Gintzler AR, Scalisi JA: Physiological correlates of multiple subtypes of enteric opiate receptor; functional analysis of myenteric receptors. Brain Research 238: 254-259, 1982.
28. Vaught JL, Cowan A, Jacoby H I: μ and , but not k, opioid agonists induce contractions of the canine small intestine in vivo. Eur J Phramacol 109: 43-48, 1985.
29. Paton WDM, Aboozar H: The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. J Physiol 194: 13-33, 1968.
30. Holzer P, Lippe ITH, Bartho l, Lembeck F: [D-Met^2, Prog^5] enkephalinamide and dynorphin- 1-13 inhibit the cholinergic contraction induced in the guinea-pig ileum by substance P. Eur J Pharmacol 91: 83-88, 1983.
31. Cherubini E, Moritak, North RA: Optic inhibition of synaptic transmission in the guinea-pig myenteric plexus. Br J Pharmacol. 85: 805-817, 1985.
32. Fosbraey P, Morton IKM, Claire M: The effect of pertussis toxin pretreatment on u-and k-opioid modulation of neurotransmitter output. Br J Pharmacol 86: 705, 1985.
33. Elmqvistd, Quastel DMJ: Presynaptic action of hemicholinium at the neuromuscular junction. J Physiol 177: 463-482, 1965.
34. Goldenberg MM: Effect of atropine and DMAE (a hemicholinium derivative) on contractile responses of the guinea-pig ileum. Can J Physiol Pharmacol 47: 185-192, 1969.
35. Erjavece F, Beaven MA, Brodie, BB: Uptake and release of ^3H-histamine in cat submaxillary gland. Fedn Proc 26: 237-240, 1967.
36. Biggs MJ, Johnson ES: Electrically-evoked release of ^3H-histamine from the guinea-pig hypothalamus. Br J Pharmacol. 70:555-560, 1980.
37. Fantozzi R, Masini E, Blandina P, Mannaioni PF, Bani-SacchiT: Release of histamine from rat mast cells by acetylcholine. Nature, 273: 473-474, 1978.
38. Blandina P, Fantozzi R, Mannaioni PF, Masini E: Characteristics of histamine release evoked by acetylcholine in isolated rat mast cells. J Physiol 301: 281-293, 1980.
39. Kaliner M, Austen KF: Immunological release of chemical mediators from human tissues. Ann Rev Pharmacol 15: 177-189, 1975.
40. Ruiz MC, Michelangel F: Evidence for a direct action of Ach on the gastric cell of the amphibian. Am J Physiol246: 16-26, 1984.
41. Ekblad EB, Licko V: A model eliciting transient responses. Am J Physiol 246: 114-121, 1984.