# Relationship Between Muscarinic Autoinhibition and the Inhibitory Effect of Morphine on Acetylcholine Release From Myenteric Plexus of Guinea Pig Ileum

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ABSTRACT—The relationship between muscarinic autoinhibition and the inhibitory effect of morphine on acetylcholine (ACh) release was investigated in a longitudinal muscle with myenteric plexus (LMMP) preparation of guinea pig ileum. Morphine (10 $\mu$M) inhibited spontaneous and evoked ACh release by electrical field stimulation (EFS) at 1 Hz but not at 10 Hz. Atropine (1 $\mu$M) did not affect the resting ACh release, but it significantly increased EFS-evoked release, suggesting activation of muscarinic autoreceptors by ACh released during EFS. Only when the autoinhibition was weakened by atropine, morphine exhibited an inhibitory effect on the EFS-evoked release at 10 Hz. Bethanechol (300 $\mu$M) inhibited the EFS-evoked release at 1 Hz but not 10 Hz, suggesting that muscarinic autoreceptors are partially or almost fully activated at 1 or 10 Hz stimulation, respectively. After bethanechol treatment, morphine did not exhibit its inhibitory effect on the EFS-evoked release at 1 Hz. Naloxone (1 $\mu$M) increased spontaneous and EFS-evoked ACh release at 1 Hz but not at 10 Hz. Following treatment with atropine, naloxone also increased ACh release at 10-Hz stimulation. These results suggest that morphine and an endogenous opioid inhibit ACh release from LMMP preparations when muscarinic autoinhibition mechanism does not fully work. This inhibitory effect of morphine is discussed in relation to the calcium sensitivity of the preparations in ACh release.

*Keywords*: Muscarinic autoinhibition, Morphine, Acetylcholine release, Myenteric plexus, Ileum (guinea pig)

It is well known that morphine, a potent narcotic analgesic, has marked effects on the function of the gastrointestinal tract in the human and experimental animals. Morphine was reported to have inhibitory effects on gastrointestinal motility (1–3). The inhibitory effect is mainly attributable to its ability to reduce acetylcholine (ACh) release from nerve terminals of enteric cholinergic neurons (4–7). Longitudinal muscle myenteric plexus (LMMP) preparations of guinea pig ileum have been widely used for studying ACh release from enteric neurons. Interestingly, the inhibitory effect of morphine on the ACh release evoked by electrical field stimulation (EFS) in the LMMP preparations was dependent on frequency of electrical pulses used for the stimulation. Morphine inhibited ACh release evoked by low frequency of stimulation more profoundly than that by high frequency (4–7). However, why the effects of morphine depended on the frequency of EFS has not been fully explained. It was reported that the enteric neurons in guinea pig intestine contained endogenous opioids such as enkephalin and dynorphine (8–10) and that these endogenous opioids, concomitantly released by EFS, were suggested to play a modulatory role on the ACh release (11).

On the other hand, there is considerable evidence to suggest that the release of ACh from the LMMP preparations is subject to autoinhibition, whereby released ACh inhibits its own release from the nerve terminals through muscarinic autoreceptors located on presynaptic plasma membranes (12–16). Therefore, it would be interesting to study the relationship between those two inhibitory modulations on the ACh release. In the present study, we investigated the relationship between the

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inhibitory effect of morphine on the EFS-evoked ACh release and the muscarinic autoinhibition and also the role of endogenous opioids in the ACh release in guinea pig LMMP preparations.

## MATERIALS AND METHODS

Male guinea pigs, weighing 300–800 g, were lightly anesthetized with diethylether and killed by bleeding. The longitudinal muscle preparations of the ileum with the myenteric plexus were made as described previously (17). The preparation was mounted in an organ bath containing 3 ml of Tyrode solution of the following composition: 136.9 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.05 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.4 mM $NaH_2PO_4$ and 5.6 mM glucose. Bathing medium was kept at 37℃ and bubbled with 95% $O_2$ and 5% $CO_2$.

The preparations were equilibrated for 15 min by perfusion with Tyrode solution containing physostigmine salicylate (5 $\mu$M) and choline chloride (1 $\mu$M) at a rate of 1–2 ml/min. Then, perfusion was stopped and the bathing medium was replaced by 3 ml of fresh Tyrode solution. After 1 min (in the case of 10-Hz stimulation) or 4 min (in the case of 1-Hz stimulation), the medium was collected for measurement of spontaneous ACh release by replacing it with fresh Tyrode solution. For the EFS-induced release of ACh, the stimulation was performed in trains of 20 sec at 10 Hz or 200 sec at 1 Hz. Bathing fluid was collected after a further 40-sec period (Fig. 1). These experimental procedures for sample collection were repeated 2 or 3 times with 15 min intervals. EFS was carried out with a pair of platinum electrodes, one at the top and the other at the bottom of the preparation. The parameters for EFS were as follows: supramaximal voltage (50 V), pulse duration of 0.5 msec, 200 pulses at 1 or 10 Hz. The first stimulation was carried out in the absence of test drug(s), and the second and third were in the presence of test drug(s). Our preliminary study showed that amounts of ACh released by EFS were fairly constant up to the 3rd trial; however, it became variable after the 4th stimulation. At the end of the experiment, the strips were blotted and weighed for expression of release as the amount of ACh per g tissue. The amount of ACh released in response to each stimulation was calculated by subtracting the release during the resting period (spontaneous release) immediately before stimulation from the total release during stimulation.

ACh released into the medium was assayed by HPLC. For analysis by HPLC, ACh in aliquots of the medium was precipitated with $KI_3$ in the presence of tetraethylammonium and 50 pmol ethylhomocholine as a coprecipitant and an internal standard, respectively. The quaternary ammonium compounds precipitated were dissolved with about 1 ml of acetonitrile, and then the solution was passed through a Bio Rad AG 1×8 anion exchange resin column (Bio-Rad Lab., Richmond, CA, USA) to trap $I_3^-$ in the solution. The effluents were evaporated to dryness under reduced pressure and the

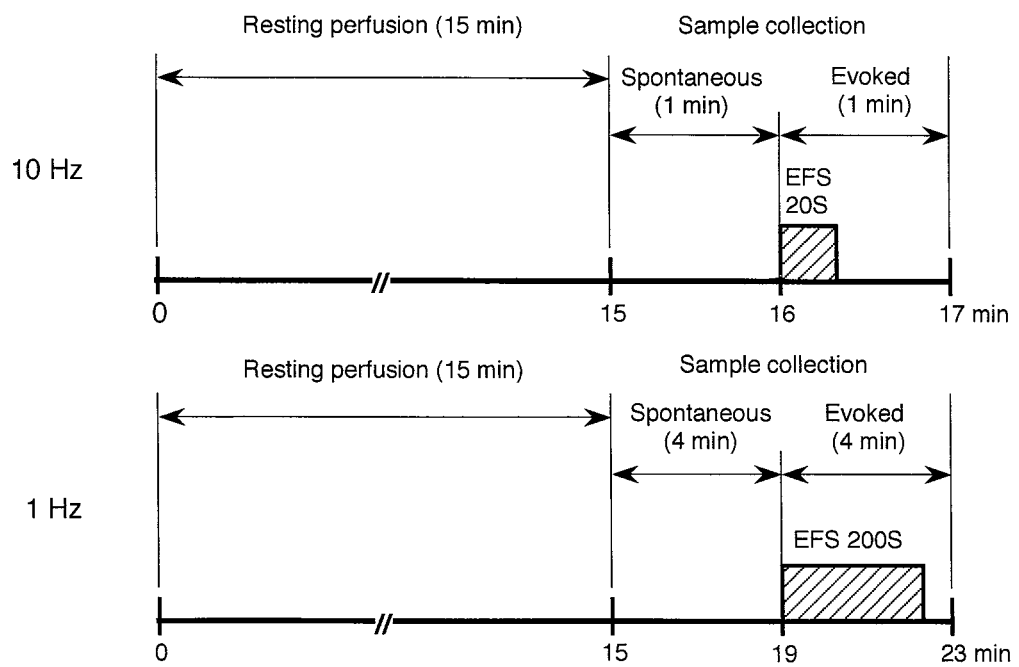


**Fig. 1.** Protocol for the experiments on ACh release.

dried samples were stored in a refrigerator until assay. At use, the dried samples were dissolved in 100 $\mu$l water and filtered through a 0.45-$\mu$m-pore-sized filter, and 50 $\mu$l of the filtrates were injected into an HPLC assay system for ACh determination. ACh was determined by a Yanaco reversed-phase HPLC (Yanaco, Kyoto), developed first by Potter et al. (18), using a postcolumn enzyme (acetylcholinesterase plus choline oxidase) reactor (Eicom AC-Enzympak; Eicom, Kyoto) instead of the enzyme-flow system of the original method.

The drugs used were morphine hydrochloride (Takeda Chemical Industries, Osaka) and naloxone hydrochloride, atropine sulfate, bethanechol hydrochloride and physostigmine salicylate (Sigma, St. Louis, MO, USA).

Statistical analyses of the data for two groups was carried out with a paired Student's *t*-test and for three groups with a one-way analysis of variance and Bonferroni's test. P values less than 0.05 were considered significant. The mean±S.E.M. was used throughout this manuscript.

## RESULTS

### *Effects of morphine on ACh release evoked by EFS from LMMP preparations of guinea pig ileum*

In the present study, all experiments were carried out in the presence of 5 $\mu$M physostigmine to prevent ACh hydrolysis. EFS at 1 and 10 Hz evoked significant increase in ACh release from the LMMP preparations of guinea pig ileum (from 691±93 to 1097±164 and from 753±158 to 2183±408 pmol/g tissue/min at 1 Hz and 10 Hz stimulation, respectively). The net amount of ACh released by EFS was calculated by subtracting the spontaneous release from the total release during EFS (Fig. 2). Morphine at a concentration of 10 $\mu$M significantly inhibited spontaneous release of ACh. Morphine also significantly inhibited ACh release evoked by EFS at 1 Hz, but it did not affect EFS-evoked ACh release at 10 Hz (Fig. 2). The inhibitory effect of morphine was antagonized by 10 $\mu$M naloxone (data are not shown).

### *Effects of atropine and bethanechol on inhibition of ACh release by morphine*

The effect of morphine on ACh release was examined in the presence of atropine or bethanechol to study the relationship between autoinhibition and the inhibitory effect of morphine.

Blockade of autoreceptors by atropine (1 $\mu$M) did not affect spontaneous release of ACh (data not shown). However, it caused a great increase in EFS-evoked ACh release both at 1 and 10 Hz (Fig. 3). The percentage of increase in ACh release induced by atropine in 1-Hz stimulation was greater than that in 10-Hz stimulation (459% and 163% increase at 1 and 10 Hz, respectively), but the net amount of ACh released by EFS at 10 Hz was about 3 times greater than that evoked at 1 Hz. Although morphine (10 $\mu$M) did not affect EFS-evoked ACh release at 10 Hz (Fig. 2), it significantly inhibited ACh release evoked by 10-Hz stimulation under the canceling of autoinhibition by atropine (Fig. 3). The inhibitory effect of morphine in the presence of atropine was observed at the concentrations of 3 $\mu$M and over (Fig. 4). The inhibitory effect of 10 $\mu$M morphine in the presence of atropine was also antagonized by 10 $\mu$M naloxone (data not shown). These results suggest that morphine inhibits EFS-evoked ACh release even at 10-Hz stimulation, if autoinhibition is blocked by atropine.

Bethanechol at 300 $\mu$M inhibited the EFS-evoked ACh release at 1 Hz by >30% but it did not affect the evoked release at 10 Hz, suggesting that muscarinic autoreceptors were fully activated at 10-Hz stimulation, but only partially at 1-Hz stimulation (Fig. 5). Interestingly, morphine at 10 $\mu$M did not inhibit EFS-evoked ACh release at 1 Hz in the presence of bethanechol, suggesting that

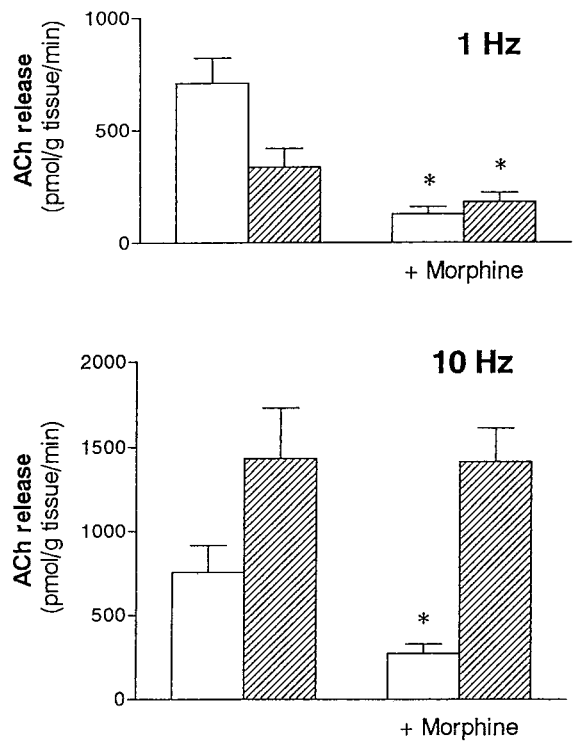


**Fig. 2.** Effects of morphine (10 $\mu$M) on spontaneous (open columns) or evoked ACh release (hatched columns) by EFS at 1 (upper panel) or 10 Hz (lower panel). Values are each the mean for 6 experiments, with S.E.M. Evoked release was expressed as the net amount of ACh released by EFS. *Significantly different from the value in the absence of morphine at $P<0.05$ (Student's *t*-test). For further details, see the Materials and Methods.

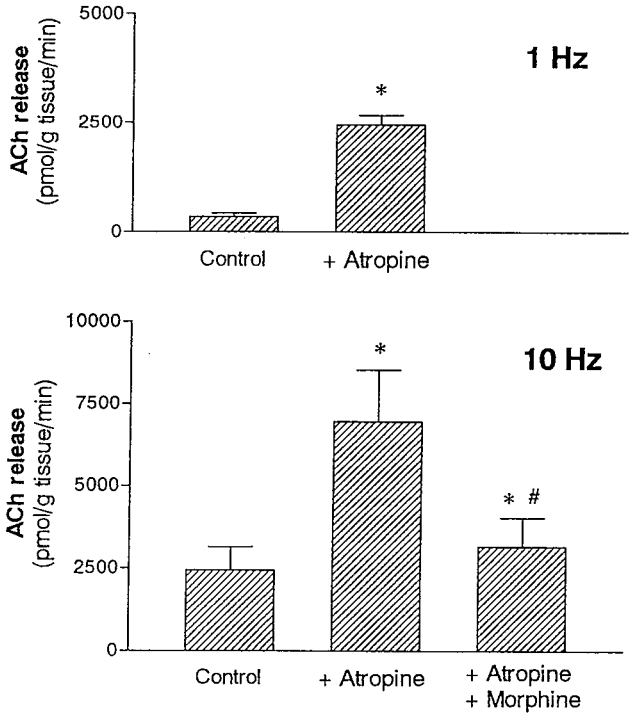


**Fig. 3.** Effects of atropine (1 $\mu$M) in the absence or presence of morphine (10 $\mu$M) on EFS-evoked ACh release at 1 (upper panel) or 10 Hz (lower panel). Values are each the mean for 6 experiments, with S.E.M. *Significantly different from the value in the absence of atropine at $P<0.05$ (Student's $t$-test and Bonferroni's test). #Significantly different from the value in the presence of atropine alone at $P<0.05$ (Bonferroni's test).

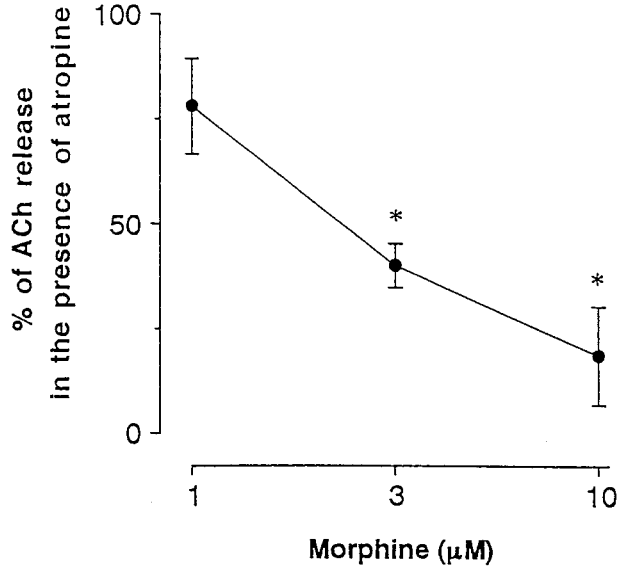


**Fig. 4.** Dose-dependent inhibitory effect of morphine on EFS-evoked ACh release at 10 Hz in the presence of atropine. Data are expressed as a percentage of the net amount of ACh evoked by EFS in the presence of atropine (1 $\mu$M). Values are each the mean for 4 to 6 experiments, with S.E.M. *Significantly different from the value in the absence of morphine at $P<0.05$ (Bonferroni's test).

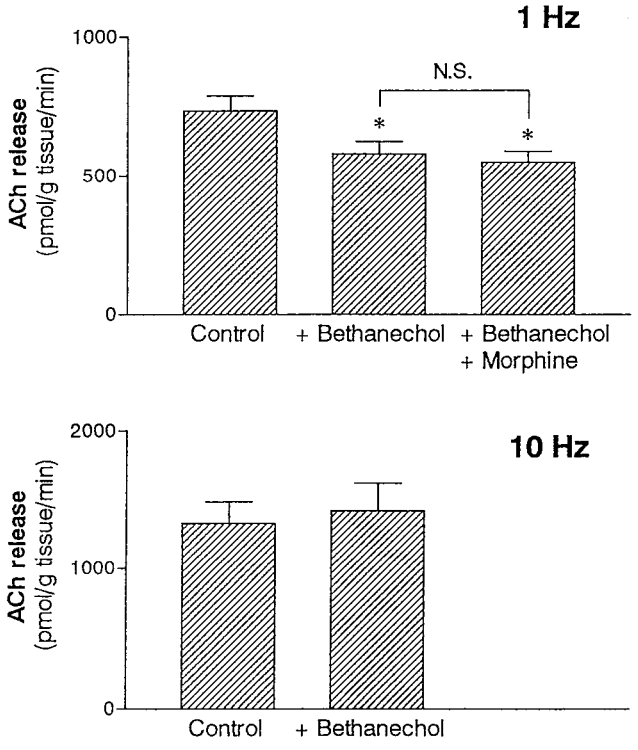


**Fig. 5.** Effects of bethanechol (300 $\mu$M) in the absence or presence of morphine (10 $\mu$M) on EFS-evoked ACh release at 1 (upper panel) or 10 Hz (lower panel). Values are each the mean for 4 to 6 experiments, with S.E.M. *Significantly different from the value in the absence of bethanechol at $P<0.05$ (Bonferroni's test). N.S.: Not significantly different between the two groups.

morphine exhibits its inhibitory effect on ACh release only when muscarinic autoreceptors are not fully activated (Fig. 5).

*Effect of naloxone on ACh release evoked by EFS*

Then the effect of naloxone on EFS-evoked ACh release was examined to study the role of endogenous opioids on ACh release. Naloxone (1 $\mu$M) significantly increased spontaneous release of ACh (Fig. 6). Naloxone also increased EFS-evoked ACh release at 1 Hz, but not at 10 Hz (Fig. 6). We further studied the effect of naloxone on ACh release in the presence of atropine to evaluate the relationship between the inhibitory effect of endogenous opioids and autoinhibition. When autoinhibition was blocked by atropine, naloxone increased ACh release evoked not only at 1 Hz but also at 10 Hz (Fig. 7). The results suggest that endogenous opioids inhibit the ACh release when muscarinic autoreceptors are not fully activated.

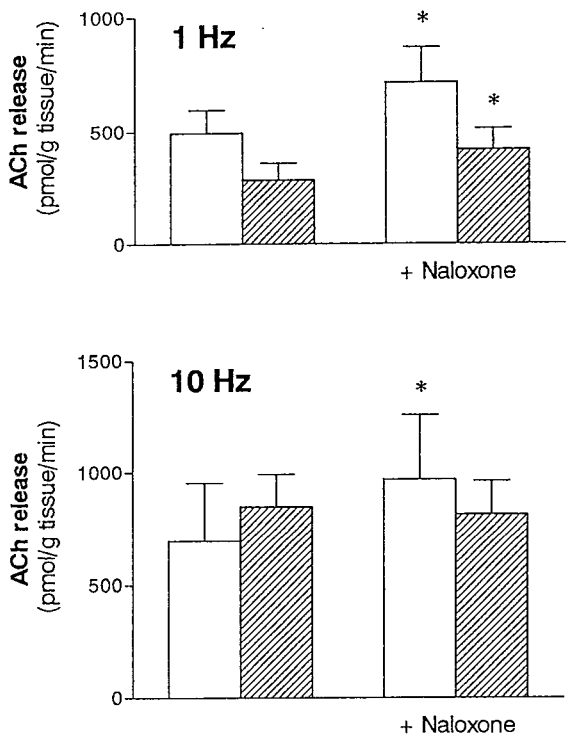


**Fig. 6.** Effects of naloxone (1 $\mu$M) on spontaneous (open columns) or EFS-evoked ACh release (hatched columns) at 1 (upper panel) or 10 Hz (lower panel). Values are each the mean for 6 to 7 experiments, with S.E.M. Evoked release was expressed as the net amount of ACh released by EFS. *Significantly different from the value in the absence of naloxone at $P<0.05$ (Student's *t*-test).

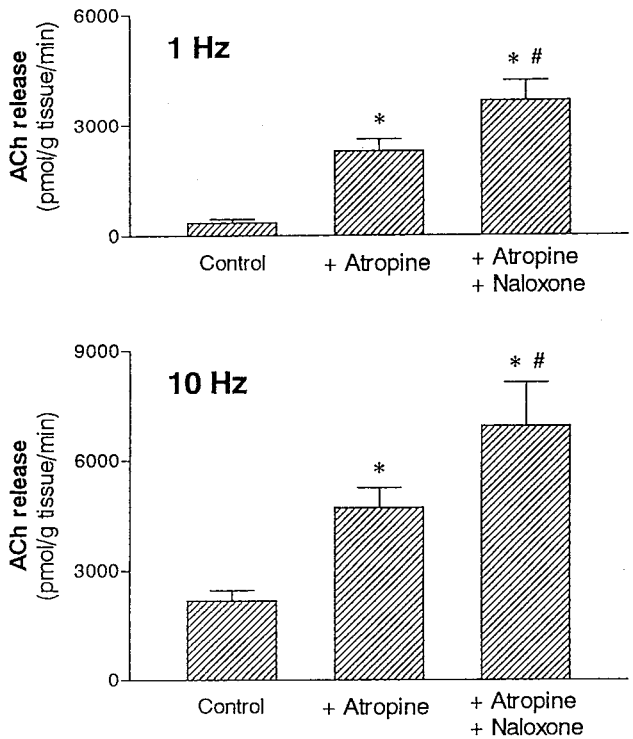


**Fig. 7.** Effects of atropine (1 $\mu$M) in the absence or presence of naloxone (1 $\mu$M) on EFS-evoked ACh release at 1 (upper panel) or 10 Hz (lower panel). Values are each the mean for 4 to 5 experiments, with S.E.M. *Significantly different from the value in the absence of atropine at $P<0.05$ (Bonferroni's test). #Significantly different from the value in the presence of atropine alone at $P<0.05$ (Bonferroni's test).

*Effect of morphine on calcium-sensitivity of LMMP preparations in ACh release in the presence or absence of atropine*

Calcium-sensitivity of LMMP preparations in EFS-evoked ACh release was studied by changing the calcium concentration in the bathing solution, $[Ca^{2+}]_o$. The lowering of $[Ca^{2+}]_o$ reduced EFS-evoked ACh release at 1 and 10 Hz, but ACh release evoked by 1-Hz stimulation was less influenced by lowering of $[Ca^{2+}]_o$ than that evoked by 10-Hz stimulation: when $[Ca^{2+}]_o$ was lowered from 1.8 mM to 0.9 mM or 0.45 mM, the reduction in 10-Hz stimulation was by 34% or 89%, respectively (Fig. 8A), while that in 1-Hz stimulation was by 9% or 73%, respectively (Fig. 8B). On the other hand, raising $[Ca^{2+}]_o$ from 1.8 mM to 3.6 mM resulted in an increase in ACh release by 15% and 35% in the 10-Hz and 1-Hz stimulation, respectively (data not shown).

When EFS was carried out at 10 Hz (autoinhibition-working condition), atropine increased $[Ca^{2+}]_o$-sensitivity of the preparations in ACh release; that is, a lower concentration of $[Ca^{2+}]_o$ was sufficient to evoke a certain level of ACh release (Fig. 8C). The result suggests that autoinhibition had decreased the $[Ca^{2+}]_o$-sensitivity under the experimental conditions. At 10-Hz stimulation, morphine itself did not affect the sensitivity (Fig. 8A), but it reversed the effect of atropine on the sensitivity (Fig. 8C): morphine decreased the sensitivity in the presence of atropine. When EFS was carried out at 1 Hz (autoinhibition-not fully working condition), morphine decreased the $[Ca^{2+}]_o$-sensitivity in ACh release (Fig. 8B).

## DISCUSSION

Morphine inhibited ACh release induced by EFS at 1 Hz but not at 10 Hz in the present study. The finding that opioid more strongly inhibited EFS-evoked ACh release at low frequency than that at high frequency was also reported previously (4–6, 19, 20). However, the reason why morphine is less effective on ACh release evoked by high frequency stimulation has not been explained so far. We presupposed that this phenomenon may relate to muscarinic autoinhibition or inhibition by endogenously released opioid. In the present report, we studied the relationship between the inhibitory effect of morphine or

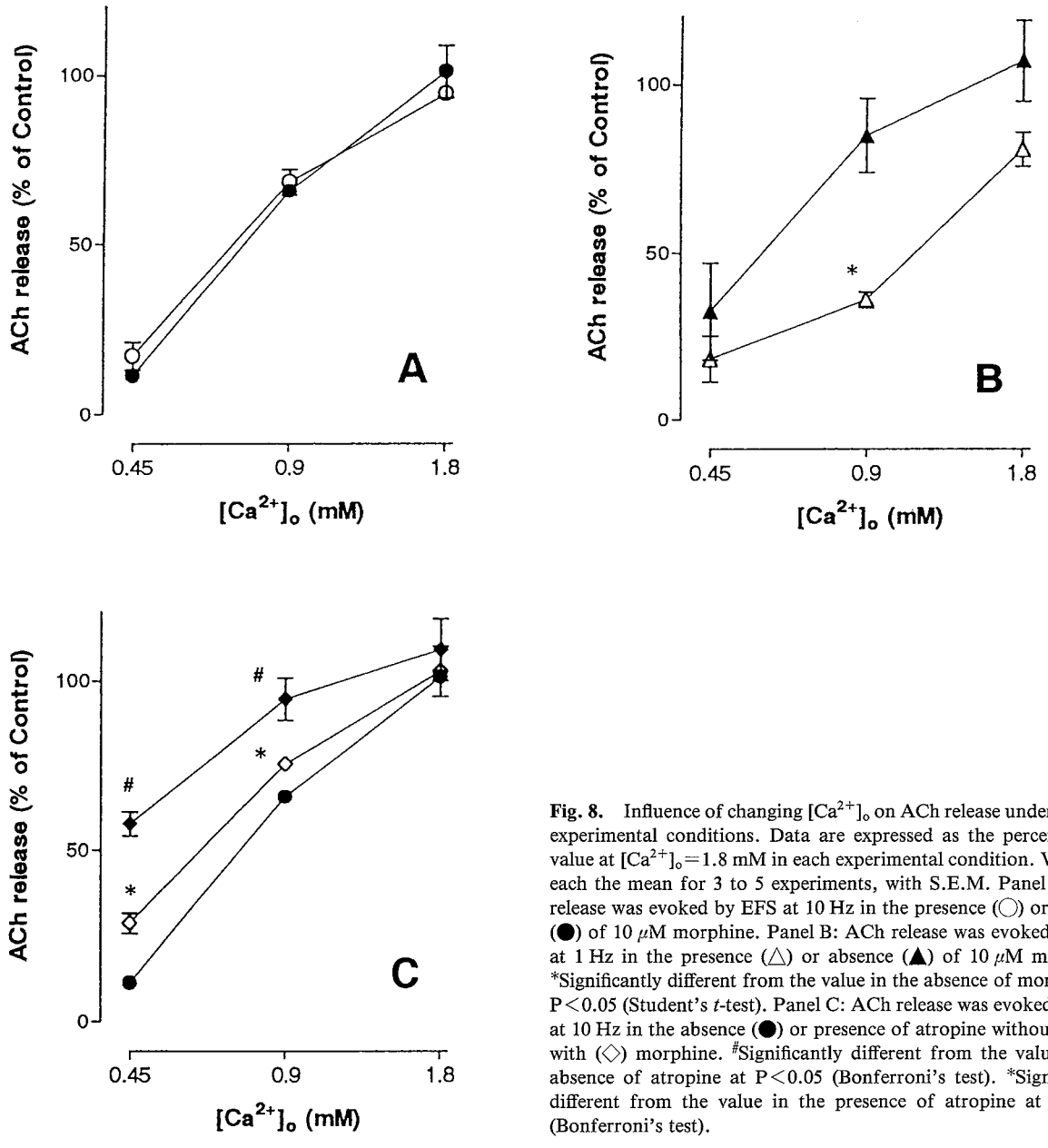


**Fig. 8.** Influence of changing $[Ca^{2+}]_o$ on ACh release under various experimental conditions. Data are expressed as the percentage of value at $[Ca^{2+}]_o = 1.8$ mM in each experimental condition. Value are each the mean for 3 to 5 experiments, with S.E.M. Panel A: ACh release was evoked by EFS at 10 Hz in the presence (○) or absence (●) of 10 $\mu$M morphine. Panel B: ACh release was evoked by EFS at 1 Hz in the presence (△) or absence (▲) of 10 $\mu$M morphine. *Significantly different from the value in the absence of morphine at $P<0.05$ (Student's $t$-test). Panel C: ACh release was evoked by EFS at 10 Hz in the absence (●) or presence of atropine without (◆) or with (◇) morphine. #Significantly different from the value in the absence of atropine at $P<0.05$ (Bonferroni's test). *Significantly different from the value in the presence of atropine at $P<0.05$ (Bonferroni's test).

endogenous opioid on ACh release and autoinhibition.

Many papers have reported that ACh release in the LMMP preparation is subjected to potent muscarinic autoinhibition (12–16). Since physostigmine was added to the Tyrode solution to quantify ACh released into medium in the present study, application of atropine to block the autoinhibition greatly increased EFS-evoked ACh release both at 1 and 10 Hz. The result indicates that the LMMP preparation was subjected to substantial autoinhibition even at 1-Hz stimulation. Interestingly, although morphine did not inhibit ACh release evoked by 10-Hz stimulation as mentioned above, it inhibited the release after the blocking the autoinhibition by atropine. These results suggest that ACh released at 1-Hz stimulation does not fully activate muscarinic autoreceptors, and that morphine has an inhibitory effect on ACh release only in the state of lack or incompleteness of muscarinic autoinhibition. Indeed, morphine inhibited EFS-evoked ACh release at 1 Hz even in the absence of atropine. Such a more potent autoinhibition caused by EFS at 10 Hz than that at 1 Hz was suggested also by Kilbinger and Wessler (21). Therefore, we tested the possibility with 300 $\mu$M bethanechol, which was a sufficient concentration to activate the autoreceptors (22). Bethanechol did not affect ACh release evoked at 10 Hz, but it caused about a 30% decrease in EFS-evoked ACh release at 1 Hz. In the

presence of bethanechol, morphine could not inhibit ACh release evoked at 1 Hz stimulation. Thus, it seems likely that autoinhibition produced by EFS at 1 Hz was incomplete and morphine exhibited further inhibition.

There is another possibility that large amount of opioids released by EFS at 10 Hz induced almost the maximum inhibition of ACh release, since it is known that many neurons within the myenteric plexus of guinea pig contain enkephalin and/or dynorphin (8–10), and that EFS releases these endogenous opioids (23, 24). The lack of an inhibitory effect of morphine on EFS-evoked ACh release at 10 Hz could be explained by this possibility. However, naloxone significantly increased EFS-evoked ACh release at 1 Hz but not at 10 Hz, indicating that this is not the case. Under blocking of the autoinhibition by atropine, naloxone further increased EFS-evoked ACh release at 10 Hz. Therefore, it seems likely that endogenous opioid is released by EFS under the present experimental conditions, and that the inhibitory effect of endogenous opioid on ACh release is closely associated with the magnitude of muscarinic autoinhibition.

Atropine increased EFS-evoked ACh release by blocking muscarinic autoinhibition, but it did not affect the spontaneous release. Since muscarinic autoreceptors might be fully activated at 10-Hz stimulation, but only partially at 1-Hz stimulation as discussed above, it seems likely that the autoreceptors are not appreciably activated at the resting state. On the other hand, morphine significantly inhibited the resting release. The result is compatible with the idea that exogenous opioid exhibits an inhibitory effect on ACh release when muscarinic autoinhibition does not fully work.

Calcium entry to the nerve terminal is the essential process for neurotransmitter release. In the present study, lowering of $[Ca^{2+}]_o$ decreased ACh release. At 10-Hz stimulation, the lowering resulted in more marked decrease in the absence of atropine (under the autoinhibition-working condition) than in the presence (under the autoinhibition-blocked condition). In other words, the mechanism of the autoinhibition decreased calcium sensitivity of the preparations in ACh release (Fig. 8C). Morphine did not affect the calcium sensitivity in the absence of atropine (Fig. 8A), but it significantly decreased the sensitivity which had been enhanced by atropine (Fig. 8C). These results suggest that activation of muscarinic autoreceptors results in a decrease in calcium sensitivity of the preparations in ACh release, and that morphine also decrease the calcium sensitivity when the autoreceptors are not fully activated. Results obtained at 1-Hz stimulation support this idea: the calcium sensitivity at 1 Hz was higher than that at 10 Hz (Fig. 8: A and B) and morphine decreased the calcium sensitivity at 1 Hz (Fig. 8B). Thus, the autoreceptors might have dominant role in inhibiting ACh release, although activation of both the autoreceptors and opioid receptors results in a decrease in calcium sensitivity of the preparations.

The present study suggests that the inhibitory effect of morphine on ACh release in LMMP preparations of guinea pig ileum is associated with the magnitude of muscarinic autoinhibition, and that morphine and probably endogenous opioid are effective under the condition in which the preparation is not subject to potent muscarinic autoinhibition. It is also suggested that the effect of morphine and muscarinic autoinhibition relate to the sensitivity of the mechanism of ACh release to $[Ca^{2+}]_o$.

REFERENCES


1 Burks TF: Mediation by 5-hydroxytryptamine of morphine stimulant action in dog intestine. J Pharmacol Exp Ther **185**, 530–539 (1973)

2 Burks TF, Hirning LD, Galligan JT and Daris TP: Motility effects of opioid peptides in dog intestine. Life Sci **31**, 2237–2240 (1982)

3 Fox JET and Daniel EE: Exgenous opiates: their local mechanisms of action in the canine small intestine and stomach. Am J Physiol **253**, G179–G188 (1987)

4 Paton WDM: The action of morphine and related substance on contraction and acetylcholine output of coaxial stimulated guinea-pig ileum. Br J Pharmacol **11**, 119–127 (1957)

5 Cox BM and Weinstock M: The effect of analgesic drugs on the release of acetylcholine from electrically stimulated guinea-pig ileum. Br J Pharmacol **27**, 81–92 (1966)

6 North RA and Tonini M: The mechanism of action of narcotic analgesics in the guinea-pig ileum. Br J Pharmacol **61**, 541–549 (1977)

7 Szerb JC: Correlation between acetylcholine release and neuronal activity in the guinea-pig ileum myenteric plexus; effect of morphine. Neuroscience **7**, 327–340 (1982)

8 Furness JB, Costa M and Miller RJ: Distribution and projections of nerves with enkephalin-like immunoreactivity in the guinea-pig small intestine. Neuroscience **8**, 653–664 (1983)

9 Vincent SR, Dalsgaard C-J, Schultzberg M, Hokfelt T, Christensson I and Terenius L: Dynorphine immunoreactive neurons in the autonomic nervous system. Neuroscience **11**, 973–988 (1984)

10 Costa M, Furness JB and Cuello AC: Separate population of opioid containing neurons in the guinea pig intestine. Neuropeptide **5**, 445–448 (1985)

11 Waterfield AA and Kosterlitz HW: Stereospecific increase by narcotic antagonists of evoked acetylcholine output in guinea-pig ileum. Life Sci **16**, 1787–1792 (1975)

12 Morita K, North RA and Tokimasa T: Muscarinic presynaptic inhibition of synaptic transmission in myenteric plexus of guinea-pig ileum. J Physiol (Lond) **333**, 141–149 (1982)

13 Alberts P, Bartfai T and Stjarne L: The effect of atropine on [$^3$H]acetylcholine secretion from guinea pig myenteric plexus evoked electrically or high potassium. J Physiol (Lond) **329**, 93–112 (1982)

14 Kilbinger H, Dietrich C and Bardeleben RSV: Functional re-

lease of presynaptic muscarinic autoreceptors. J Physiol (Lond) **86**, 77–81 (1992)

15 Saitoh N, Nishio H, Takeuchi T and Hata F: Characterization of antimuscarinic effect of cimetropium bromide in guinea pig ileum. J Smooth Muscl Res **33**, 1–9 (1997)

16 Saitoh N, Fujimoto R, Ishii T, Nishio H, Takeuchi T and Hata F: Muscarinic autoinhibition and modulatory role of protein kinase C in acetylcholine release from the myenteric plexus of guinea pig ileum. Jpn J Pharmacol **74**, 155–163 (1997)

17 Takeuchi T, Okuda M and Yagasaki O: The differential contribution of endogenous prostaglandins to the release of acetylcholine from myenteric plexus of the guinea-pig ileum. Br J Pharmacol **102**, 381–385 (1991)

18 Potter PE, Meek JL and Neff HN: Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J Neurochem **41**, 188–194 (1983)

19 Vizi ES, Ono K, Adam-Vizi V, Duncalf D and Foldes FF: Presynaptic inhibitory effect of Met-Enkephalin on [$^{14}$C]acetylcholine release from the myenteric plexus and its interaction with muscarinic negative feed back inhibition. J Pharmacol Exp Ther **230**, 493–499 (1984)

20 Kojima Y, Takahashi T, Fujina M and Owyang C: Inhibition of cholinergic transmission by opiates in ileal myenteric plexus is mediated by kappa receptor. Involvement of regulatory inhibitory G protein and calcium N-channels. J Pharmacol Exp Ther **268**, 965–970 (1993)

21 Kilbinger H and Wessler I: The variation of acetylcholine release from myenteric neurones with stimulation frequency and train length. Naunyn Schmiedebergs Arch Pharmacol **324**, 130–133 (1983)

22 Soejima O, Katsuragi T and Furukawa T: Opposite modulation by muscarinic M1 and M3 receptors of acetylcholine release from guinea pig ileum as measured directly. Eur J Pharmacol **249**, 1–6 (1993)

23 Glass J, Chan WC and Gintzler AR: Direct analysis of the release of methionine-enkephalin from guinea-pig myenteric plexus: Modulation by endogenous opioides and exogenous morphine. J Pharmacol Exp Ther **239**, 742–747 (1986)

24 Gintzler AR and Xu H: Different G proteins mediate the opioid inhibition on enhancement of evoked [5-methionine]enkephalin release. Proc Natl Acad Sci USA **88**, 4741–4745 (1991)