Jpn. J. Pharmacol. 82, 343 – 349 (2000)

Possible Role of Potassium Channels in Mu-receptor-Mediated Inhibition and Muscarinic Autoinhibition in Acetylcholine Release From Myenteric Plexus of Guinea Pig Ileum

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Received November 22, 1999 Accepted January 20, 2000

ABSTRACT—It is known that mu-agonists inhibit electrical field stimulation (EFS)-evoked ACh release from longitudinal muscle myenteric plexus (LMMP) preparation of guinea pig ileum when muscarinic autoinhibition does not fully work. In the present study, the possible role of K^+ channels in the mechanisms of mu-agonists-induced inhibition and autoinhibition of ACh release was studied. In the presence of atropine, which blocks the autoinhibition, non-selective K^+ channel blockers, tetraethylammonium (TEA) and 4-aminopyridine (4-AP), reversed the inhibitory effect of mu-agonists, morphine and [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol] enkephalin, on EFS-evoked ACh release, but not that of kappa-agonist U-50,488. Apamin, iberiotoxin or glibenclamide did not affect the inhibition of ACh release by morphine. On the other hand, in the absence of atropine (under the autoinhibition working condition), 4-AP increased EFS-evoked ACh release, but atropine did not further increase ACh release in the presence of 4-AP. In contrast, although TEA did not affect EFS-evoked ACh release, atropine increased ACh release in the presence of TEA. These results suggest that the inhibitory effects of mu-agonists and muscarinic autoinhibition on the ACh release are associated with activation of different types of K^+ channels in the guinea pig LMMP preparations: the former is associated with 4-AP- and TEA-sensitive K^+ channels and the latter is associated with 4-AP- but not TEA-sensitive K^+ channels.

Keywords: K^+ channel, Mu-receptor, Muscarinic autoinhibition, Acetylcholine release, Myenteric plexus

The opioid receptors are classified into several types, mainly mu-, kappa- and delta-receptors (1, 2) which are present in the enteric neurons (3 – 6). Longitudinal muscle myenteric plexus (LMMP) preparations of guinea pig ileum have been widely used for studying ACh release from enteric neurons. Several studies indicated that activation of mu- and kappa-receptors by exogenous opioid agonists resulted in inhibition of ACh release from the preparations, while delta-receptors play a minor role in ACh release (7, 8). The ACh release from the LMMP preparations is subject to muscarinic autoinhibition (9 – 11). We recently reported that endogenous opioid(s) inhibits ACh release by activating mu- but not kappa- or delta-receptor in LMMP of guinea pig ileum and that the inhibition of ACh release by endogenous opioid(s) become apparent only when the muscarinic autoinhibition mechanism does not fully work (12, 13). It is suggested that activation of mu-receptors in the enteric neurons leads to an increase in K^+ conductance of the cell membrane (14, 15). Morphine and other mu-agonists inhibited spontaneous firing of membrane potentials in the myenteric neurons of guinea pig ileum (16). In the cell soma of enteric neurons, morphine and [Met^5] enkephalin hyperpolarized the resting membrane potential (17) and increased Ca^{2+}-dependent after-hyperpolarization following to action potentials (18). These effects were suggested to be due to enhancement of K^+ conductance (14, 15). The enhancement of K^+ conductance results in a decease in neurotransmitter release by reducing the invasion probability of an impulse propagating along the nerve axon into nerve terminals or shortening duration of action potential, which reduces Ca^{2+} influx into nerve terminals probably due to hyperpolarization of the membrane potentials (19). Potassium channels are now classified into more than ten types based on their elec-

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trophysiological and pharmacological properties (20). Several types of K^+ channels including calcium-activated K^+ channels (K_{Ca} channels) and adenosine 5′-triphosphate (ATP)-sensitive K^+ channels (K_{ATP} channels) are known to be present on plasma membrane in central and peripheral nervous systems and to play a role in modulation of neurotransmission (for a review, see Ref. 21). However, there is little information regarding the type of K^+ channels involved in the inhibition of ACh release via mu-receptors in the LMMP preparations of guinea pig ileum. Therefore, in the present work, we studied the effects of various K^+ channel blockers on mu-agonist-induced inhibition of ACh release evoked by EFS to determine the type of K^+ channels involved in the inhibition. We also studied whether the K^+ channels involved in mu-receptor-mediated inhibition is associated with muscarinic autoinhibition.

MATERIALS AND METHODS

Male guinea pigs, weighing 300 – 400 g, were sacrificed by bleeding. The LMMP preparations of the ileum were made as described previously (22). The preparation was mounted in an organ bath containing 3 ml of Tyrode solution. The bathing medium was kept at 37°C 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 μM) and choline chloride (1 μM) 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, the medium was collected for measurement of resting ACh release by replacing it with fresh Tyrode solution. Electrical field stimulation (EFS) was carried out with a pair of platinum electrodes in trains of 20 s at 10 Hz, for the EFS-evoked release of ACh, and then the bathing fluid was collected after a further 40-s period. These experimental procedures for sample collection were repeated 2 or 3 times with 15-min intervals between each. At the end of the experiment, the strips were blotted and weighed for expression of ACh release as pmol per g tissue. The amount of ACh released by 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 in the medium was assayed by HPLC as described elsewhere (12). Unless otherwise stated, all experiments except the experiment to study the effect of K^+ channel blockers on muscarinic autoinhibition were carried out in the presence of 1 μM atropine

The drugs used were morphine hydrochloride (Takeda Chemical Industries, Osaka); naloxone hydrochloride, atropine sulfate, 4-aminopyridine (4-AP), tetraethylammonium (TEA), physostigmine salicylate (Wako Pure Chemical, Osaka); glibenclamide, [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol] enkephalin (DAMGO), U-50,488 methane sulfonate (Funakoshi, Tokyo); apamin, iberiotoxin (Peptide Institute, Osaka).

Statistical analyses of the data for two groups were carried out with the 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

Reverse by 4-AP and TEA of inhibitory effect of morphine on ACh release evoked by EFS from LMMP preparations of guinea pig ileum

EFS at 10 Hz evoked a significant increase in ACh release from the LMMP preparations. Morphine at 10 μM significantly inhibited EFS-evoked ACh release at 10 Hz in the presence of 1 μM atropine as previously shown (12) (Fig. 1A). The inhibitory effect of morphine was completely antagonized by the selective mu-receptor antagonist cyprodime at 0.1 μM, indicating that morphine inhibited ACh release via activation of mu-receptors: morphine inhibited the release from 4284 ± 253 pmol·g tissue^{-1}·min^{-1} (n = 4) to 3086 ± 225, and cyprodime reversed the reduced release to 4446 ± 218. Therefore, we used morphine as a mu-agonist in the following experiments. In the presence of 1 μM atropine, non-selective K^+ channel blockers 4-AP at 1 mM and TEA at 1 mM increased EFS-evoked ACh release (Fig. 1A) and reversed the ACh release inhibition by morphine (Fig. 1B).

Effects of 4-AP and TEA on inhibition of ACh release by opioid agonists

We next examined the effects of 4-AP and TEA on inhibitory effects of another mu-agonist DAMGO (1 μM) and a kappa-agonist U-50,488 (1 μM) on ACh release evoked by EFS to characterize the type of K^+ channels activated by mu-agonists. DAMGO and U-50,488 at 1 μM significantly inhibited EFS-evoked ACh release at 10 Hz in the presence of 1 μM atropine as shown previously (13). The inhibitory effect of DAMGO was completely reversed by 4-AP or TEA (Fig. 2). On the other hand, the inhibitory effect of U-50,488 was not sufficiently reversed by these K^+ channel blockers.

Effects of apamin, iberiotoxin and glibenclamide on inhibition of ACh release by morphine

We further studied the type of K^+ channels involved in mu-receptor-mediated inhibition of ACh release using a small-conductance K_{Ca} channel (SK_{Ca} channel) blocker, apamin (23); a large-conductance K_{Ca} channel (BK_{Ca} channel) blocker, iberiotoxin (24); and a K_{ATP} channel blocker,

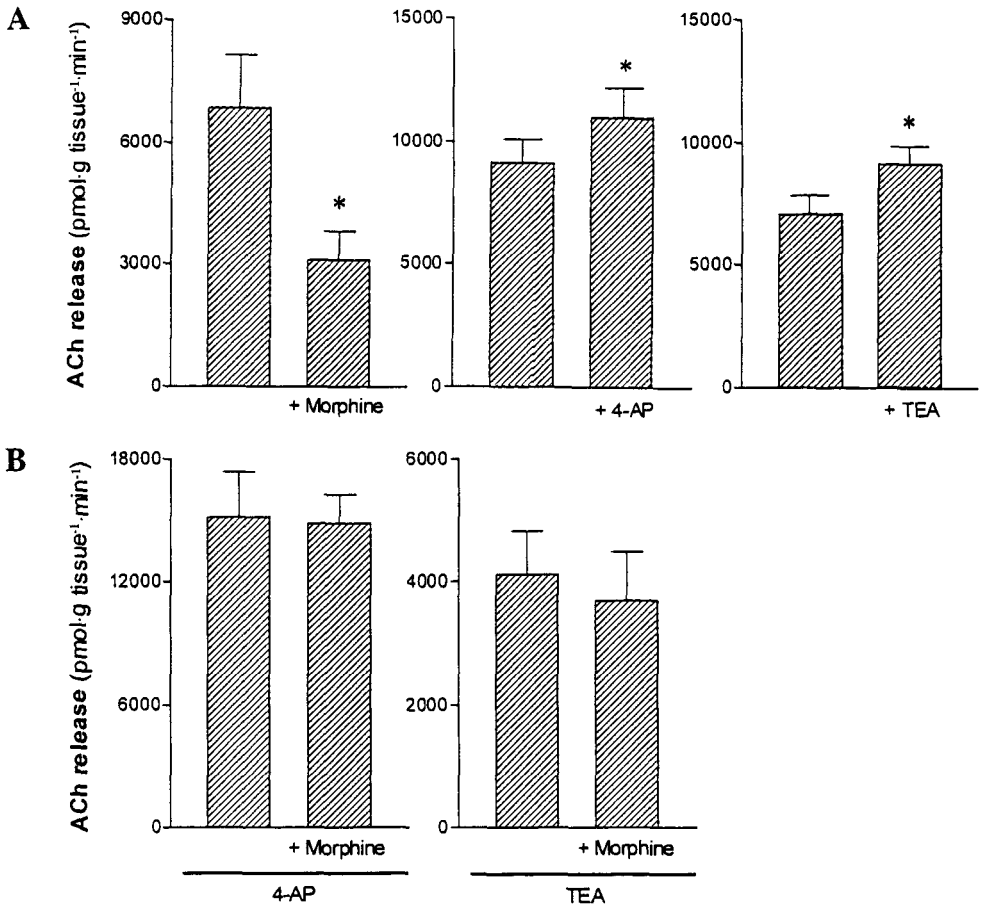

Fig. 1. Effects of 4-AP and TEA on the morphine-induced inhibition of ACh release from LMMP preparation of guinea pig ileum. A: ACh release was evoked by EFS at 10 Hz in the absence or presence of 10 μM morphine, 1 mM 4-AP or TEA. B: Morphine-induced inhibition of ACh release was examined in the presence of 1 mM 4-AP or TEA. Atropine (1 μM) was added throughout the experiments. Values are each the mean for 4 to 6 experiments, with S.E.M. Evoked release was expressed as the net amount of ACh release 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.

glibenclamide (25). None of these K^+ channel blockers affected the inhibition of ACh release by morphine, indicating that SK_{Ca}, BK_{Ca} and K_{ATP} channels are not involved in the inhibition of ACh release via mu-receptor activation (Fig. 3).

Relation of increase in ACh release by naloxone to K^+ channel blockade

Next, we studied whether K^+ channels are involved in the inhibition of ACh release by endogenous opioids. Blockade of the action of endogenous opioids by naloxone at 1 μM increase in EFS-evoked ACh release (12). Interestingly, the increase in ACh release by naloxone was not seen in the presence of 1 mM 4-AP or TEA that increased the ACh release via K^+ channel blockade, suggesting that K^+ channels involved in the endogenous mu-agonist-mediated inhibition are 4-AP- and TEA-sensitive (Fig. 4).

Effects of 4-AP and TEA on ACh release in the absence and presence of atropine

The inhibition of ACh release via mu receptor is affected by muscarinic autoinhibition (12). Therefore, we examined whether K^+ channels are also associated with the muscarinic autoinhibition in ACh release. In the absence of atropine, namely, under the muscarinic autoinhibition-working condition, 4-AP or TEA increased or failed to increase EFS-evoked ACh release by itself, respectively (Fig. 5). However, in the presence of 4-AP or TEA, atropine (1 μM) did not further increase or increased ACh release, respectively (Fig. 5), suggesting that K^+ channels involved in the autoinhibition are 4-AP- but not TEA-sensitive. Apamin, iberiotoxin and glibenclamide did not increase EFS-evoked ACh release in the absence of atropine (data not shown).

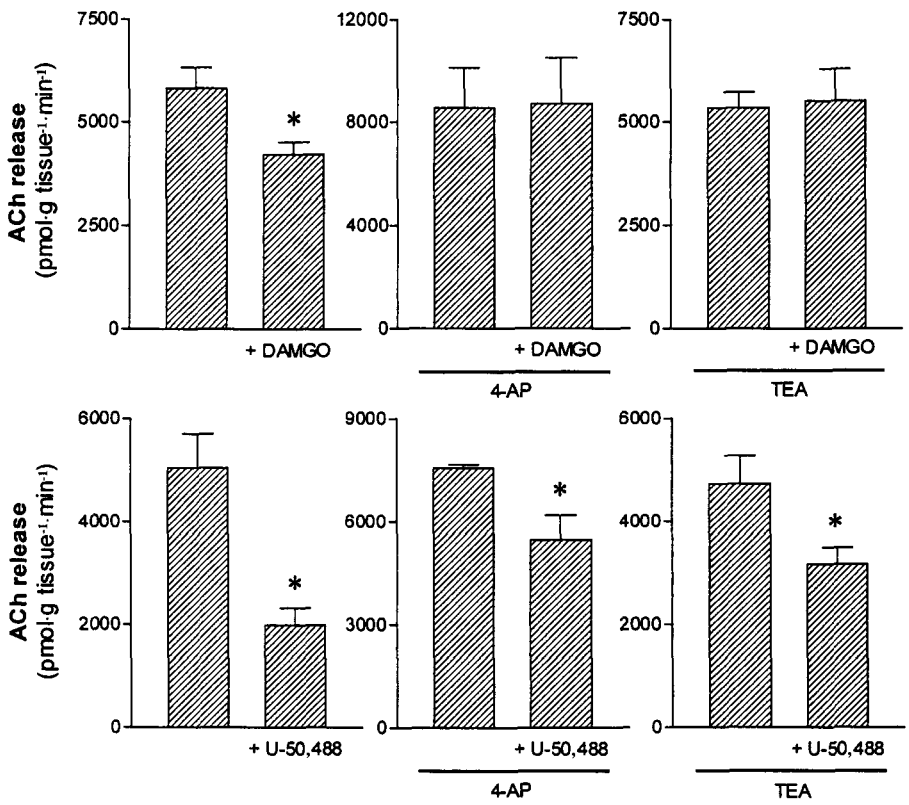

Fig. 2. Effects of 4-AP and TEA on the DAMGO- or U-50,488-induced inhibition of ACh release from LMMP preparation of guinea pig ileum. ACh release was evoked by EFS at 10 Hz in the absence or presence of 1 μM DAMGO (upper panels) or U-50,488 (lower panels). The experiments were also carried out in the presence of 1 mM 4-AP or TEA. Atropine (1 μM) was added throughout the experiments. Values are each the mean for 3 to 4 experiments, with S.E.M. *Significantly different from the value in the absence of DAMGO or U-50,488 at $P<0.05$ (Student's t-test). Value expressed as inhibition rate (% inhibition) by U-50,488 in the presence of 4-AP or TEA is significantly different at $P<0.05$ from the value of U-50,488 alone (Bonferroni's test).

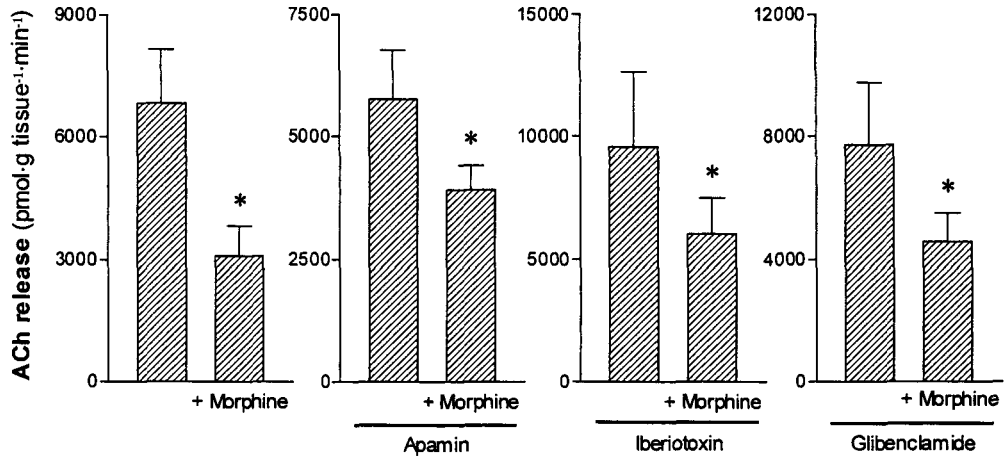

Fig. 3. Effects of apamin, iberiotoxin and glibenclamide on the morphine-induced inhibition of ACh release from LMMP preparation of guinea pig ileum. ACh release was evoked by EFS at 10 Hz in the absence or presence of morphine (10 μM). The experiments were also carried out in the presence of apamin (0.1 μM), iberiotoxin (0.1 μM) or glibenclamide (1 μM). Atropine (1 μM) was added throughout the experiments. 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 $P<0.05$ (Student's t-test). There was no significant difference among the values expressed as inhibition rate (% inhibition) by morphine in the absence and presence of apamin, iberiotoxin or glibenclamide (Bonferroni's test).

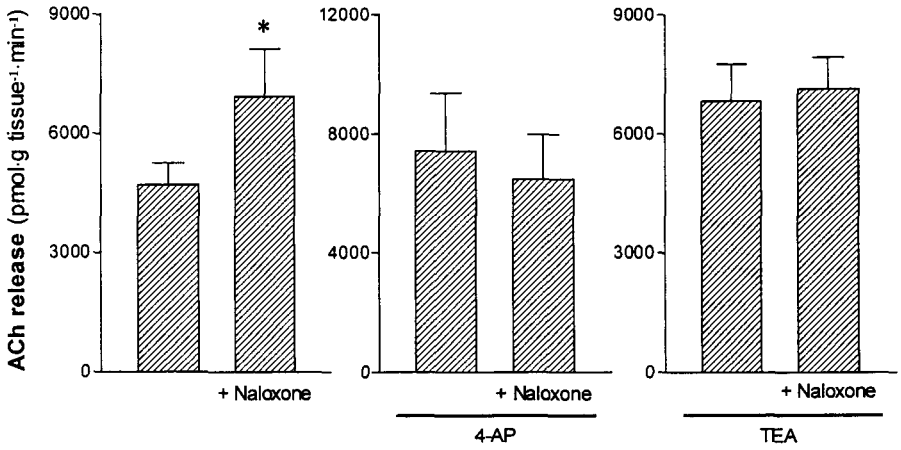

Fig. 4. Effects of 4-AP and TEA on the naloxone-induced increase in ACh release from LMMP preparation of guinea pig ileum. ACh release was evoked by EFS at 10 Hz in the absence of presence of naloxone (1 μM). The experiments were also carried out in the presence of 1 mM 4-AP or TEA. Atropine (1 μM) was added throughout the experiments. Values are each the mean for 4 to 5 experiments, with S.E.M. *Significantly different from the value in the absence of naloxone at $P<0.05$ (Student's t-test). There was no significant difference between the values of naloxone in the absence and presence of 4-AP or TEA (Student's t-test).

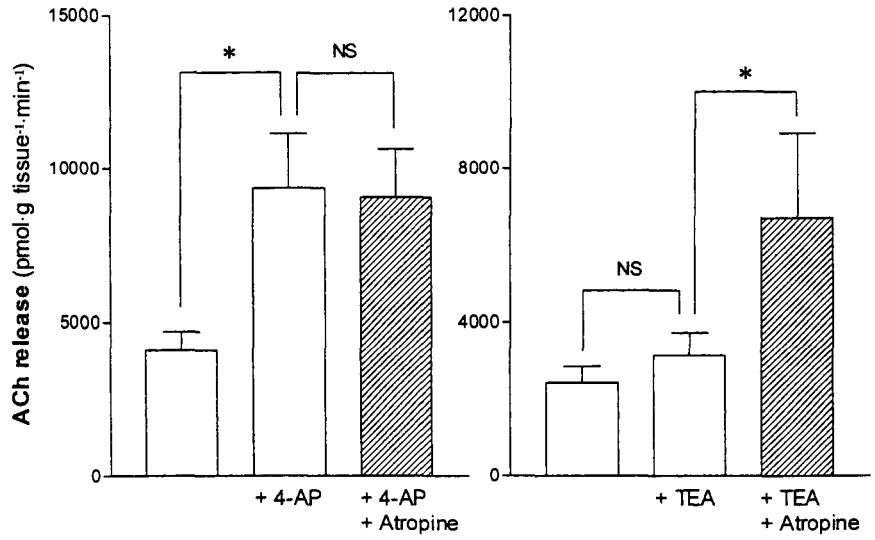

Fig. 5. Effects of 4-AP and TEA without or with atropine on EFS-evoked ACh release from LMMP preparation of guinea pig ileum. ACh release was evoked by EFS at 10 Hz in the absence or presence of 1 mM 4-AP or TEA without or with 1 μM atropine. Values are each the mean for 4 experiments, with S.E.M. *Significantly difference between the two values at $P<0.05$ (Bonferroni's test).

DISCUSSION

The inhibitory effect of morphine via activating mu-receptors on ACh release evoked by EFS in LMMP preparations of guinea pig ileum was reversed by non-selective K^+ channel blockers 4-AP and TEA. These blockers also reversed the inhibitory effect of another mu-receptor selective agonist DAMGO. The effects of 4-AP on the inhibition by mu-receptor agonists is consistent with the results obtained by using other mu-receptor agonists (normorphine, Met^5-enkephalin and Leu^5-enkephalin) (26). These results suggest that 4-AP- and TEA-sensitive K^+ channels are involved in the inhibition of ACh release via activation of mu-receptors. On the other hand, U-50,488, a kappa-receptor-selective agonist, inhibited EFS-evoked ACh release even in the presence of 4-AP and TEA. In enteric neurons, it is suggested that activation of mu-receptors results in an increase in K^+ conductance of the cell membrane, while activation of kappa-receptors results in a decrease in voltage-dependent Ca^{2+} conductance (14, 15).

Therefore, it seems likely that the difference in reversibility by the non-selective K^+ channel blockers between two kinds of inhibition of ACh release induced by morphine/DAMGO (mu-agonist) and U-50,488 (kappa agonist) are due to different intracellular mechanisms activated by mu- and kappa-receptors.

We suggested that the inhibitory effect via activation of mu-receptors on ACh release was associated with magnitude of muscarinic autoinhibition in the LMMP of guinea pig ileum. That is, morphine and DAMGO inhibited ACh release evoked by EFS when muscarinic autoinhibition did not fully work (13). Therefore, there is a possibility that 4-AP and TEA reversed the inhibitory effect of morphine and DAMGO by affecting the muscarinic autoinhibition. However, this is unlikely to be the case, because the effects of 4-AP and TEA were studied under the condition in which muscarinic autoreceptors were blocked by atropine in the present study.

The presence of SK_{Ca}, BK_{Ca} and K_{ATP} channels on the plasma membrane of nerve cells (21) and their roles in the opioid-induced modulation of neurotransmission have been reported (27 – 30). An inhibitory effect of morphine on substance P release evoked by antidromic stimulation of the sciatic nerve in rat hind paw was suggested to be mediated by activation of SK_{Ca} and BK_{Ca} (27). Involvement of BK_{Ca} channels in DAMGO-induced inhibition of cholinergic neurotransmission was suggested in guinea pig and human airway (28). K_{ATP} channels are suggested to be involved in the antinociceptive effect of morphine (29, 30). However, apamin, iberiotoxin and glibenclamide did not reverse morphine-induced inhibition of ACh release in the present study. These results suggest that SK_{Ca}, BK_{Ca} and K_{ATP} channels are not associated with the mechanism of mu-receptor-mediated inhibition of ACh release in LMMP preparation of guinea pig ileum.

We previously showed that in the presence of atropine, the selective mu-receptor antagonist cyprodime as well as the nonselective opioid antagonist naloxone increased ACh release from the LMMP preparations, whereas a selective kappa-receptor antagonist nor-binaltorphimine did not affect ACh release (13). Furthermore, putative endogenous ligands for the mu-receptor, endomorphin-1 and -2, inhibited ACh release in the LMMP preparations, and the inhibitory effects were completely antagonized by cyprodime (31). These results suggest that an endogenous opioid(s) acts on mu-receptors to modulate ACh release in the LMMP preparations. In the present study, we also examined the involvement of K^+ channels in the modulation of ACh release by endogenous opioid(s). Both 4-AP and TEA significantly increased ACh release evoked by EFS by themselves in the presence of atropine (Fig. 1). However, 4-AP and TEA did not further increase the increased ACh release by naloxone, and naloxone did not further increase the increased ACh release by 4-AP or TEA (Fig. 4). Therefore, it is likely that 4-AP- and TEA-sensitive K^+ channels are involved in the mechanism of ACh release inhibition by endogenous opioid(s) via mu-receptors.

Various intracellular responses induced by activation of muscarinic receptors are intimately associated with alteration in ion conductance of cell membrane. Activation of muscarinic receptors caused an increase or decrease in potassium conductance, an increase in cation (most importantly, sodium) conductance and a decrease in calcium conductance (see for review, Ref. 32). An increase in membrane K^+ conductance of the plasma membrane by muscarinic agonists was reported in autonomic ganglion cells (33, 34) and central neurons (35). The properties of K^+ current increased by muscarinic agonists were very similar to those by mu-receptor agonists (32). As mentioned above, activation of mu-receptors results in inhibition of ACh release when muscarinic autoinhibition does not fully work (13). Thus, the correlation of the muscarinic autoinhibition and the mu-receptor-mediated inhibition seems interesting. In fact, 4-AP alone increased EFS-evoked release of ACh. In the presence of 4-AP, atropine did not further increase ACh release (Fig. 5). Similar results were also reported in the rat frontal cortical slices (36). By contrast, TEA alone did not affect ACh release, while atropine significantly increased ACh release in the presence of TEA (Fig. 5). These results suggest that mechanism of muscarinic autoinhibition is associated with 4-AP- but not TEA-sensitive K^+ channels in the LMMP preparations of guinea pig ileum. Thus, activation of mu-receptors and muscarinic autoreceptors leads to activation of a different type of K^+ channels. Activation of K^+ channels results in hyperpolarization of membrane potential, which in turn depresses neurotransmitter release by blockade of action potential propagation to the nerve terminals or shortening of action potential that decrease Ca^{2+} influx. It is possible that the intracellular pathway of the inhibition of ACh release via activation of the different type of K^+ channels are overlapping.

In summary, the present results suggest that the inhibitory effects of exogenous and endogenous opioids on ACh release via activation of mu-receptors are mediated by opening of 4-AP- and TEA-sensitive K^+ channels in the LMMP preparations of guinea pig ileum, while that via activation of muscarinic autoreceptors is mediated by opening of 4-AP- but not TEA-sensitive K^+ channels.

REFERENCES

1 Lord JAH, Waterfield AA, Hughes J and Kosterlitz HW: Endogenous opioid peptides: Multiple agonists and receptors. Nature **267**, 495 - 499 (1977)

2 Martin WR: Pharmacology of opioids. Pharmacol Rev **35**, 283 - 323 (1983)

3 Chavkin C and Goldstein A: Demonstration of a specific dynorphin receptor in guinea pig ileum myenteric plexus. Nature **291**, 591 - 593 (1981)

4 Corbett AD, Paterson SJ, McKnight AT, Magman J and Kosterlitz HW: Dynorphin 1 - 8 and dynorphin 1 - 9 are ligands for the (-subtype of opiate receptor. Nature **299**, 79 - 81 (1982)

5 Goldstein A and James IF: Site-directed alkylation of multiple opioidreceptors. II. Pharmacological selectivity. Mol Pharmacol **25**, 343 - 348 (1984)

6 Leslie FM, Chavkin C and Cox BM: Opioid binding properties of brain and peripheral tissues: Evidence for heterogeneity in opioid ligand binding sites. J Pharmacol Exp Ther **214**, 395 - 411 (1980)

7 Fox AJ and Morton IKM: Mu and kapper opioid receptor modulation of 5HT3 and NK-3 receptor-evoked release of acetylcholine from the guinea-pig ileum myenteric plexus. Naunyn Schmiedebergs Arch Pharmacol **344**, 8 - 15 (1991)

8 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)

9 Alberts P, Bartfai T and Stjärne 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)

10 Kilbinger H, Dietrich C and Bardeleben RSV: Functional release of presynaptic muscarinic autoreceptors. J Physiol (Paris) **87**, 77 - 81 (1993)

11 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 myenteric plexus of guinea pig ileum. Jpn J Pharmacol **74**, 155 - 163 (1997)

12 Nishiwaki H, Saitoh N, Nishio H, Takeuchi T and Hata F: Relationship between muscarinic autoinhibition and the inhibitory effect of morphine on acetylcholine release from myenteric plexus of guinea pig ileum. Jpn J Pharmacol **77**, 271 - 278 (1998)

13 Nishiwaki H, Saitoh N, Nishio H, Takeuchi T and Hata F: Relationship between inhibitory effect of endogenous opioid via mu-receptors and muscarinic autoinhibition in acetylcholine release from myenteric plexus of guinea pig ileum. Jpn J Pharmacol **77**, 279 - 286 (1998)

14 Cherubini E and North RA: μ and κ opioids inhibit transmitter release by different mechanisms. Proc Natl Acad Sci USA **82**, 1860 - 1863 (1985)

15 Noth RA: Opioid receptor types and membrane ion channels. Trends Neurosci **9**, 114 - 117 (1986)

16 North RA and Williams JT: Extracellular recording from the guinea-pig myenteric plexus and the action of morphine. Eur J Pharmacol **45**, 23 - 33 (1977)

17 Morita K and North RA: Opiate activation of potassium conductance in myenteric neurons: inhibition by calcium ion. Brain Res **242**, 145 - 150 (1982)

18 Cherubini E, Morita K and North RA: Morphine augments calcium-dependent potassium conductance in guinea-pig myenteric neurones. Br J Pharmacol **81**, 617 - 622 (1984)

19 Illes P: Mechanisms of receptor-mediated modulation of transmitter release in noradrenergic, cholinergic and sensory neurones. Neuroscience **17**, 909 - 928 (1986)

20 Sanguinetti MC and Spector PS: Potassium channelopathies. Neuropharmacology **36**, 755 - 762 (1997)

21 Aronson JK: Potassium channels in nervous tissue. Biochem Pharmacol **43**, 11 - 14 (1992)

22 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)

23 Banks BE, Brown C, Burgess GM, Burnstock G, Claret M, Cocks TM and Jenkinson DH: Apamin blocks certain neurotransmitter-induced increases in potassium permeability. Nature **282**, 415 - 417 (1979)

24 Galvez A, Gimenez-Gallego G, Reuben JP, Roy-Contancin L, Feigenbaum P, Kaczorowski GJ and Garcia ML: Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion *Buthus tamulus*. J Biol Chem **265**, 11083 - 11090 (1990)

25 Niki I, Kelly RP, Ashcroft SJ and Ashcroft FM: ATP-sensitive K-channels in HIT T15 beta-cells studied by patch-clamp methods, 86Rb efflux and glibenclamide binding. Pflugers Arch **415**, 47 - 55 (1989)

26 Foldes FF, Ohta Y, Shiwaku Y, Vizi ES, von Dijk TT and Morita K: The influence of 4-aminopyridine on parasympathetic transmission. *In* Advances in Bioscience, Vol **35**, Aminopyridines and Similarly Acting Drugs, Edited by Lechat P, Thesleff S and Bowman WC, pp 127 - 139, Pergamon Press, Oxford (1982)

27 Yonehara N and Takiuchi S: Involvement of calcium-activated potassium channels in the inhibitory prejunctional effect of morphine on peripheral sensory nerves. Regul Pept **68**, 147 - 153 (1997)

28 Miura M, Belvisi MG, Stretton CD, Yacoub MH and Barnes PJ: Role of K^+ channels in the modulation of cholinergic neural responses in guinea-pig and human airways. J Physiol (Lond) **455**, 1 - 15 (1992)

29 Ocana M, Del Pozo E and Baeyens JM: ATP-dependent K^+ channel blockers antagonize morphine- but not U-50,488H-induced antinociception. Eur J Pharmacol **230**, 203 - 207 (1993)

30 Welch SP and Dunlow LD: Antinociceptive activity of intrathecally administered potassium channel openers and opioid agonists: a common mechanism of action? J Pharmacol Exp Ther **267**, 390 - 399 (1993)

31 Nishiwaki H, Saitoh N, Nishio H, Takeuchi T and Hata F: Inhibitory effect of endomorphin-1 and -2 on acetylcholine release from myenteric plexus of guinea pig ileum. Jpn J Pharmacol **78**, 83 - 86 (1998)

32 Noth RA: Muscarinic receptors and membrane ion conductance. Trends Pharmacol Sci **7**, Suppl 19 - 22 (1986)

33 Dodd J and Horn JP: Muscarinic inhibition of sympathetic C neurones in the bullfrog. J Physiol (Lond) **334**, 271 - 291 (1983)

34 Hartzell HC, Kuffler SW, Stickgold R and Yoshikami D: Synaptic excitation and inhibition resulting from direct action of acetylcholine on two types of chemoreceptors on individual amphibian parasympathetic neurones. J Physiol (Lond) **271**, 817 - 846 (1977)

35 Egan TM and North RA: Acetylcholine hyperpolarizes central neurones by acting on an M2 muscarinic receptor. Nature **319**, 405 - 407 (1986)

36 Töröcsik A and Vizi ES: 4-Aminopyridine interrupts the modulation of acetylcholine release mediated by muscarinic and opiate receptors. J Neurosci Res **27**, 228 - 232 (1990)