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β-Agonists Modulate ACh-Inhibition of a K Current in Intestinal Smooth Muscle Cells

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ACh causes a long-lasting inhibition of STOCs via G proteins in intestinal smooth muscle cells. We examined the effects of isoproterenol (Iso) on the ACh-induced inhibition of STOCs in isolated ileal smooth muscle cells using the GΩ-seal whole cell clamp technique. In control, ACh (1 μM) completely suppressed STOCs, which did not desensitize over a period lasting 20 minutes. When Iso (10 μM) was added to the bath in the presence of ACh, the ACh-induced inhibition of STOCs was gradually removed. This effect of Iso was prevented by propranolol (10 μM). Application of Db-cAMP (500 μM) mimicked the Iso effects. Intracellulary applied GTP-γS (100 μM) gradually suppressed STOCs in the absence of ACh, which could not be removed by either Iso or Db-cAMP. These results suggest that β-adrenergic stimulation causes a removal of the muscarinic inhibition of STOCs via a cAMP-dependent process.

Cholinergic and β-adrenergic stimulations are major physiological mechanisms in the regulation of the intestinal tone (9). ACh, an excitatory neurotransmitter, causes an increase of Ca^{2+} influx which results in contraction of intestinal smooth muscle. Electrophysiologically, ACh causes the activation of a non-selective cation conductance and the inhibition of an oscillatory Ca^{2+}-activated K^+ current in the ileal smooth muscle cells (1,2,11), thereby depolarizing the cells which may facilitate the voltage-dependent Ca^{2+} influx. On the other hand, β-adrenergic stimulation causes an increase of intracellular

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Abbreviations: ACh, acetylcholine; STOCs, spontaneous transient outward currents (oscillatory Ca^{2+}-activated K^+ currents); DB-cAMP, dibutyryl cyclic AMP.

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cAMP resulting in the relaxation of intestinal smooth muscle (10). The effects of β-agonists on the membrane currents of intestinal smooth muscle cells are unknown. Since the intestinal tone is regulated by both cholinergic and β-adrenergic receptors which exert opposite effects, we hypothesized that β-agonists may modulate the ACh-induced suppression of STOCs in the ileal smooth muscle cell. In the present study, we examined the effects of isoproterenol and dibutyryl cAMP on the ACh-induced suppression of STOCs. We found that β-adrenergic stimulation causes a removal of the ACh-effect. These results suggest a novel mechanism for the regulation of intestinal tone by neurotransmitters.

Materials and Methods

The longitudinal smooth muscle cells of rabbit ileum were dispersed by the following procedures based on the method described by Ohya et al (13). Albino rabbits of either sex (1.5-2.0 kg) were anesthetized by an intravenous injection of pentobarbital sodium (50 mg/kg). The distal ileum was excised and longitudinal smooth muscle layers were peeled from the underlying circular muscle. Small pieces of the tissue were incubated in a Ca^{2+}-free incubation solution for 30 minutes at 37°C under mild shaking. After the incubation, smooth muscle cells were dispersed by gentle agitation at 4°C in Ca^{2+}-free physiological salt solution. The dispersed cells were stored in the minimum essential medium Eagle with Hank's balanced salt solution at 4°C for later use. All experiments were performed at 33-35°C within 4 hours after preparation.

The GΩ-seal patch clamp technique was used in the whole cell configuration (8). A heat-polished patch pipette, filled with an artificial internal solution (for composition, see below) had a tip resistance of 5-7 MΩ. Membrane currents and membrane potential were monitored with a high-gain storage oscilloscope (COS5020-ST, Kikusui Electronic, Tokyo, Japan). The current amplifier used was a List EPC-7 (FRG). The data were stored in a video cassette recorder using the PCM converter system (NF electronic circuit design, Tokyo, Japan, RP-880), and were replayed onto a recorder for illustration and analysis. At the start of each experiment, the series resistance was compensated. The composition of the control bathing solution (the standard perfusion solution) was as follows (in mM): NaCl 136, KCL 6, $CaCl_2$ 1.8, $MgCl_2$ 1.0, D-glucose 5.5, HEPES-NaOH buffer 10 (pH 7.4). In addition to the nominal Ca^{2+}-free bathing solution, the Ca^{2+}-free incubation solution contained 1 mg/ml collagenase (Wako, Tokyo), 1 mg/ml trypsin inhibitor (Sigma, type S-1), 2 mg/ml bovine serum albumin. In the whole cell experiments, the pipettes were filled with the internal solution containing (in mM): KCl 140, $MgCl_2$ 2, EGTA 0.15, Na_2ATP 3, Na_2GTP 0.1, HEPES-KOH buffer 10 (pH 7.20).

Iso, Propranolol and GTP-γS were purchased from Sigma (St.Louis, MO, USA).

Results

Figure 1a shows the effects of ACh on the oscillatory Ca^{2+}-activated K^+ current measured from a dispersed ileal smooth muscle cell. To avoid contamination by the non-selective cation channel current induced by ACh (1), the cells were voltage-clamped at 0 mV in the present study. ACh (1 μM)

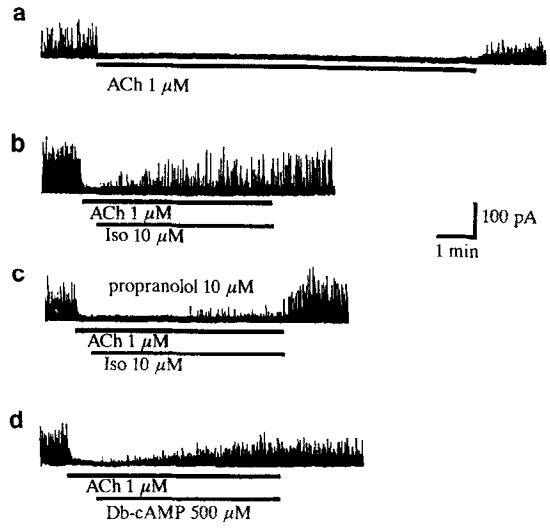

Figure 1

a. The effects of acetylcholine (ACh) on the oscillatory Ca^{2+}-activated K^+ current (STOCs) in the control condition. To avoid contamination by the non-selective cation channel current induced by ACh (1), the cells were voltage-clamped at 0 mV in the present study. When ACh (1 μM) was applied to the bathing solution, I_o was transiently enhanced and then rapidly suppressed. After washout of ACh, STOCs gradually returned to control levels.
b. The effects of the β-adrenergic agonist, isoproterenol (Iso, 10 μM), on the ACh-induced suppression of I_o. Iso was applied to the bathing solution after the complete suppression of STOCs. In the presence of ACh and Iso, STOCs gradually reappeared.
c. The effects of the β-adrenergic receptor antagonist, propranolol (10 μM). Propranolol affected neither STOCs nor ACh-induced suppression of STOCs, but in the presence of propranolol, Iso failed to remove the ACh-induced suppression of STOCs.
d. The effects of dibutyryl cyclic AMP (Db-cAMP, 500 μM) on the ACh-induced suppression of STOCs. Db-cAMP was applied to the bathing solution after ACh completely suppressed STOCs. In the presence of Db-cAMP, STOCs gradually reappeared.

transiently accelerated and then rapidly suppressed STOCs. The magnitude of transient enhancement of STOCs varied from cell to cell and was often negligible in our experimental conditions. In the continued presence of ACh, the ACh-induced suppression of STOCs was maintained over a period lasting 20 minutes (n=20). After washout of ACh, STOCs gradually returned to control levels. Figure 1b shows the effects of the β-adrenergic agonist, isoproterenol (Iso, 10 μM), on the ACh-induced suppression of I_o. Iso was added to the bathing solution after ACh completely suppressed STOCs. In the presence of ACh and Iso, STOCs gradually reappeared (n=10). We then conducted the entire experiments in the presence of propranolol (10 μM), a β-adrenergic receptor antagonist (Fig. 1c). Propranolol affected neither STOCs in the control conditions nor the ACh-induced suppression of

STOCs. In the presence of propranolol, isoproterenol failed to remove the ACh-induced suppression of STOCs. Since β-adrenergic stimulation causes an increase in intracellular cAMP in smooth muscle cells (10), we examined the effects of Db-cAMP on the ACh-induced suppression of STOCs(Fig. 1d). Db-cAMP (500 μM) was added to the bathing solution after ACh completely suppressed STOCs. STOCs gradually reappeared in the presence of Db-cAMP. Reappearance of STOCs followed a similar time course in both the Iso and Db-cAMP experiments (n=5).

These results indicate that β-adrenergic stimulation removes the ACh-inhibition of STOCs via a cAMP-dependent process in ileal smooth muscle.

ACh-induced suppression of STOCs involves multiple steps in the signalling. Muscarinic ACh receptors, GTP-binding protein (G protein) and Phospholipase C (PLC) are some of the units involved in the ACh-induced suppression of STOCs (2,5,6,7). β-adrenergic modulation of the ACh-inhibition of STOCs could, therefore, occur at a variety of sites in the signalling.

We examined the effects of Iso (10 μM) on the ACh-induced suppression of STOCs in GTP-γS-loaded ileal smooth muscle cells. Intracellulary applied GTP-γS (100 μM) caused a persistent inhibition of STOCs, and on the other hand, GDP-βS (500 μM) prevented the ACh-induced inhibition of STOCs (not shown), suggesting that a certain subpopulation of G proteins are involved in the signalling. Under persistent activation of the G protein by GTP-γS, neither Iso nor Db-cAMP restored STOCs.

Even when Iso was applied before (for 2-3 minutes) and during perfusion with ACh, STOCs followed the time course shown in Fig. 2b. Initially, STOCs was suppressed by ACh, then it gradually reappeared in the presence of Iso. The same observations were obtained when the cells were pre-incubated in a solution containing 500 μM Db-cAMP (n=4, not shown). Thus, Iso or Db-cAMP failed to prevent the initiation of the ACh-induced inhibition of STOCs. The time course of reappearance of STOCs in the Iso or Db-cAMP-treated cells was quite similar to that observed when Iso and Db-cAMP

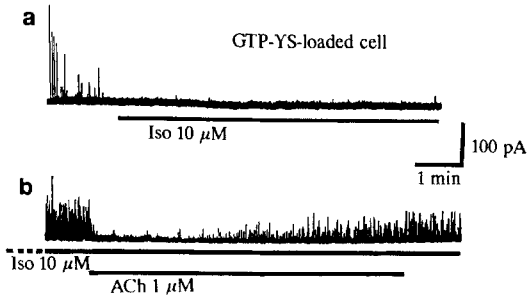

Figure 2

a. The effects of Iso on the GTP-γS (100 μM)-induced suppression of STOCs. GTP-γS caused a persistent inhibition of STOCs and Iso failed to recover STOCs.
b. The ACh-induced suppression of STOCs in Iso-pretreated cells. Initially STOCs was suppressed by ACh, then it gradually reappeared in the presence of Iso (10 μM).

were applied after the appearance of ACh-inhibition of STOCs (Fig. 1b). These results suggest that the modulation process on ACh-effects induced by Iso requires ACh binding to the muscarinic receptors.

Discussions

The major findings of the present study are as follows. 1; Isoproterenol removes or desensitizes the ACh-induced inhibition of STOCs in ileal smooth muscle cells. 2; The effects of isoproterenol are mediated by β-adrenergic receptors via an intracellular cAMP-dependent process. 3; The isoproterenol-induced removal of ACh-inhibition of STOCs did not occur in GTP-γS-loaded cells. 4; Agonist-binding to the muscarinic receptor is probably essential for the initiation of the modulation process. The ACh-induced inhibition of STOCs involves muscarinic receptors, G proteins and PLC. Activation of PLC releases IP_3, which releases Ca^{2+} from intracellular stores (4). IP_3-induced depletion of submembranous Ca^{2+} may be involved in the inhibition of STOCs (5). The Iso-induced modulation itself can occur at various sites in this multistep process. Since neither Iso nor Db-cAMP could remove the inhibition of STOCs in GTP-γS-loaded cells, the site of Iso-induced modulation probably exists upstream of the G protein-PLC-channel coupled pathway. Therefore, β-adrenergic stimulation effects the functions of the muscarinic receptors and/or the interaction between the receptor and G proteins. Since the initiation of the ACh-induced suppression and the time-course of the removal of the suppression were unaffected by preapplication of either Iso or Db-cAMP, the desensitization process

must start after application of ACh to the cells. Agonist-binding to the muscarinic receptors may be essential for β-adrenergic receptor-mediated removal of ACh-inhibition of STOCs. The desensitization of muscarinic receptors can be caused by β-adrenergic receptor kinase (β-ARK) in heart cell. β-ARK can only phosphorylate agonist-bound receptors, thus converting them to the desensitized state (3,12). Thus the present results suggest that similar mechanisms may underlie the β-adrenergic receptor-induced removal of ACh-inhibition of STOCs in ileal smooth muscle cells. The effects of β-adrenergic agonists on the ACh-induced inhibition of STOCs presented in this study may play an important role in the cholinergic and adrenergic regulation of intestinal tone.

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