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Imipramine Inhibits A-type Delayed Rectifier and ATP-Sensitive K^+ Currents Independent of G-Protein and Protein Kinase C in Murine Proximal Colonic Myocytes

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The effects of imipramine on A-type delayed rectifier K^+ currents and ATP-sensitive K^+ (K_{ATP}) currents were studied in isolated murine proximal colonic myocytes using the whole-cell patch-clamp technique. Depolarizing test pulses between -80 mV and +30 mV with 10 mV increments from the holding potential of -80 mV activated voltage-dependent outward K^+ currents that peaked within 50 ms followed by slow decreasing sustained currents. Early peak currents were inhibited by the application of 4-aminopyridine, whereas sustained currents were inhibited by the application of TEA. The peak amplitude of A-type delayed rectifier K^+ currents was reduced by external application of imipramine. The half-inactivation potential and the half-recovery time of A-type delayed rectifier K^+ currents were not changed by imipramine. With 0.1 mM ATP and 140 mM K^+ in the pipette and 90 mM K^+ in the bath solution and a holding potential of –80 mV, pinacidil activated inward currents; this effect was blocked by glibenclamide. Imipramine also inhibited K_{ATP} currents. The inhibitory effects of imipramine in A-type delayed rectifier K^+ currents and K_{ATP} currents were not changed by guanosine 5-O-(2-thiodiphosphate) (GDPβS) and chelerythrine, a protein kinase C inhibitor. These results suggest that imipramine inhibits A-type delayed rectifier K^+ currents and K_{ATP} currents in a manner independent of G-protein and protein kinase C.

Key words: A-type delayed rectifier K^+ currents, ATP-sensitive K^+ currents, Imipramine, Colonic myocytes

INTRODUCTION

Imipramine is a tricyclic antidepressant drug used in the treatment of depression and affective disorders associated with mood disturbances. However, imipramine has been classified as a class Ia cardiac antiarrhythmic agent (Valenzuela *et al.*, 1994) and is also used as a therapeutic agent for the altered motility in irritable bowel syndrome (Gorard *et al.*, 1995). Cardiac arrhythmia and gastrointestinal dysmotility are associated with alterations of membrane ionic conductance. Imipramine reportedly acts on a variety of ion channels. For example, imipramine inhibited voltage-dependent Na^+, Ca^{2+} and K^+ currents in ventricular myocytes and neurons (Isenberg and Tamargo, 1985; Ogata *et al.*, 1989; Choi *et al.*, 1992; Wooltorton and Mathie, 1993; Pancrazio *et al.*, 1998; Kuo, 1998). It is therefore possible that imipramine may also affect ion channels in gastrointestinal smooth muscle cells. Also, fluoxetine, another antidepressant agent, was reported to block the delayed rectifier K^+ channels in canine and human jejunal circular smooth muscle cells (Farrugia, 1996).

K^+ channels control the contraction of gastrointestinal smooth muscles by setting resting potential and influencing slow wave and action potential configuration. Several types of K^+ channels are expressed in gastrointestinal smooth muscles, including Ca^{2+}-activated K^+ channels, ATP-sensitive K^+ (K_{ATP}) channels, inwardly rectifying K^+ channels, and delayed rectifier K^+ channels (Kuriyama *et al.*, 1998). The delayed rectifier K^+ channels are important in the regulation of colonic smooth muscle electrical

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activity because they provide outward currents over the voltage range in which these tissues operate (Koh *et al.*, 1999). K_{ATP} channels stabilize the resting membrane potential and cell excitability. A common feature of these channels is that they are activated by a decrease in the intracellular ATP concentration. The synthetic K^+ channel openers, such as lemakalim, cromakalim, pinacidil and diazoxide, activate K_{ATP} channels, whereas glibenclamide and tolbutamide specifically inhibit K_{ATP} channels (Edward and Weston, 1993). The aim of this study was to explore whether imipramine could modulate delayed rectifier K^+ currents and K_{ATP} currents in proximal colonic myocytes.

MATERIALS AND METHODS

Isolation of murine colonic myocytes

Colonic myocytes were isolated from 20- to 30-day-old Balb/C mice of either sex. Mice were anesthetized with chloroform and sacrificed by cervical dislocation, and the proximal colon was quickly removed. The colon was opened along the myenteric border, and the mucosa and submucosa were removed in Ca^{2+}-free Hanks solution containing (in mM): 125 NaCl, 5.36 KCl, 15.5 NaOH, 0.336 Na_2HCO_3, 0.44 KH_2PO_4, 10 glucose, 2.9 sucrose, 11 HEPES. The solution was adjusted to pH 7.4 with Tris. Strips of colonic muscle were transferred to the same solution with 0.1% collagenase (Worthington Biochemical Co.), 0.2% fatty acid-free bovine serum albumin (Sigma Chemical Co.), 0.1% trypsin inhibitor (Sigma Chemical Co.), and 0.01% papain (Sigma Chemical Co.). Incubation in the enzyme solution was carried out at 37°C for 10~12 min, then the tissues were washed with Ca^{2+}-free Hanks solution. Single cells were obtained by gentle agitation with wide-bored glass pipette. Isolated cells were kept at 4°C until use. Before electrophysiological experiments, a drop of the suspension was pipetted into a small chamber (0.3 mL) on the stage of an inverted microscope. All experiments were carried out within 6 h of cell dispersion and performed at room temperature.

Membrane current recording

The standard whole-cell patch-clamp technique was used to record membrane currents (Hamil *et al.*, 1981). Glass pipettes with resistances of 3~5 MΩ were used. Membrane currents were amplified by an Axopatch 1-D (Axon Instruments) and command pulses were applied using an IBM-compatible computer and pClamp software v.6.0 (Axon Instruments). The data were filtered at 5 kHz and displayed on an oscilloscope, a computer monitor and a pen recorder (Recorder 220, Gould).

Solutions and drugs

For the recording of delayed rectifier K^+ currents, the internal pipette solution contained (in mM): 10 NaCl, 102 KCl, 1 $CaCl_2$, 1 GTP, 10 HEPES, 10 EGTA, 5 ATP, 1 $MgCl_2$; this solution was adjusted to pH 7.2 by KOH (38 mM). The external bath solution contained (in mM): 125 NaCl, 5.36 KCl, 1 $MgCl_2$, 5 glucose, 1.8 $MnCl_2$; this solution was adjusted to pH 7.4 by Tris. For the recording of K_{ATP} currents, the internal pipette solution contained (in mM): 10 NaCl, 102 KCl, 1 $CaCl_2$, 1 GTP, 10 HEPES, 10 EGTA, 5 ATP, 1 $MgCl_2$; this solution was adjusted to pH 7.2 by KOH (38 mM). The external bath solution contained (in mM): 52 NaCl, 90 KCl, 1 $MgCl_2$, 0.2 $CaCl_2$; this solution was adjusted to pH 7.4 by Tris.

Chelerythrine was purchased from RBI. Imipramine, 4-aminopyridine (4-AP), GDPβS, tetraethylammonium (TEA), pinacidil, and glibenclamide were purchased from Sigma.

Statistical analysis

The results are expressed as means ± SE. n is the number of cells tested. Statistical significance was evaluated by Student's t test. P values less than 0.05 were considered significant.

RESULTS

Delayed rectifier K^+ currents

To minimize Ca^{2+}-activated K^+ channels, we used an internal solution containing 10 mM EGTA, while the bath solution contained 1.8 mM $MnCl_2$. Depolarizing test pulses between -80 mV and +30 mV with 10 mV increments from the holding potential of -80 mV activated voltage-dependent outward currents that peaked within 50 ms followed by slow decreasing sustained currents (Fig. 1A). 4-AP (5 mM) reduced the amplitude of early peak currents (Fig. 1B). Fig. 1C shows the current traces at +10 mV of holding potential in the absence and presence of 4-AP. The subtraction currents in Fig. 1D show that 4-AP inhibited the early peak currents. These 4-AP-sensitive currents have been described as A-type delayed rectifier K^+ currents (Koh *et al.*, 1999). On the other hand, TEA (10 mM) reduced the amplitude of sustained currents without affecting the amplitude of early peak currents (Fig. 2A and B). The subtraction currents in Fig. 2C show that TEA inhibited only the sustained currents. Fig. 2D shows the current-voltage relationship at the early peak in the absence and presence of TEA.

Effects of imipramine on the A-type delayed rectifier K^+ currents

Imipramine inhibited A-type delayed rectifier K^+ currents in a concentration-dependent manner. When the inhibitory effect of imipramine was measured, approximately 47% inhibition was observed at 10 μM (Fig. 3A and B). To explore G-protein involvement in the inhibitory action of imipramine,

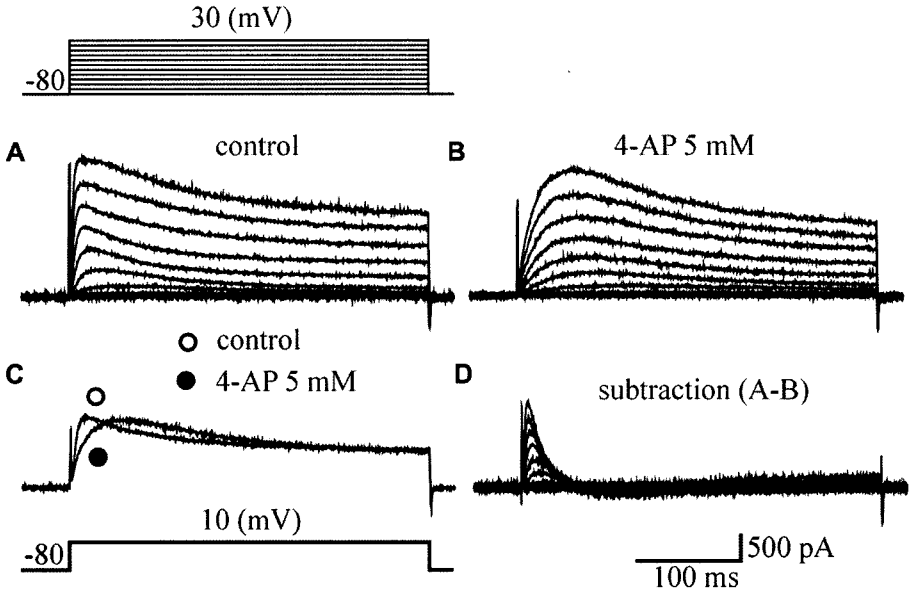

Fig. 1. Effects of 4-AP on the delayed rectifier K^+ currents. Membrane potential was stepped from -80 mV to the test potential between -80 and +30 mV in 10 mV increments. (A), control. (B), in the presence of 4-AP (5 mM). (C), the current traces at the +10 mV steps in the absence and in the presence of 4-AP. (D), difference currents obtained by subtracting currents obtained in the presence of imipramine from control current.

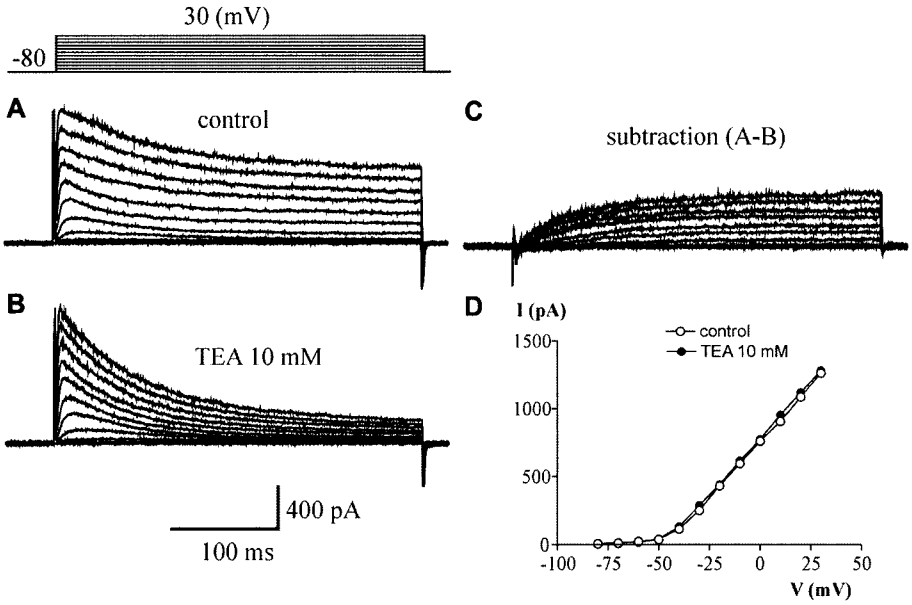

Fig. 2. Effects of TEA on the delayed rectifier K^+ currents. Membrane potential was stepped from -80 mV to test potential between -80 and +30 mV in 10 mV increments. (A), control. (B), in the presence of TEA (10 mM). (C), difference currents obtained by subtracting currents obtained in the presence of TEA from control currents. (D), current-voltage relationship for the early peak currents in the absence and presence of TEA.

the effects of GDPβS, a non-hydrolyzable guanosine 5-diphosphate (GDP) analogue that permanently inactivates GTP-binding proteins (Komori *et al.*, 1993), were tested. As shown in Fig. 4A and B, imipramine-induced inhibition of A-type delayed rectifier K^+ currents was not changed by intracellular application of GDPβS (1 mM). To test whether the activation of protein kinase C by imipramine inhibits A-type delayed rectifier K^+ currents, protein kinase C inhibitor was added. Chelerythrine is a potent protein kinase C inhibitor that is non-competitive with respect to ATP (Nixon, 1997). Following pretreatment with chelerythrine (1 μM) for 20 min, the inhibition of A-type delayed rectifier K^+ currents by imipramine was not changed (Fig. 4C and D). Chelerythrine itself had no effects on the A-type delayed rectifier K^+ currents (not shown).

To study the voltage dependency of steady-state inacti-

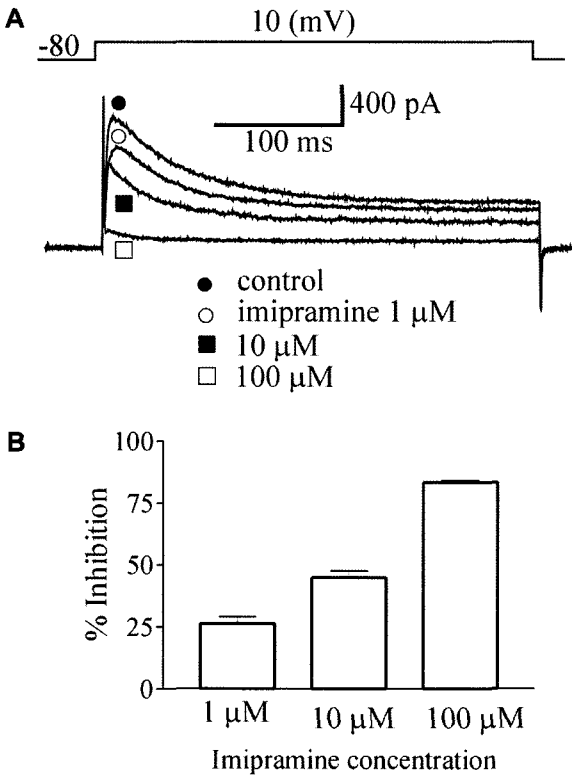

Fig. 3. Effects of imipramine on the A-type delayed rectifier K^+ currents. (A) Membrane potential was stepped from -80 to 0 mV in the control and in the presence of various concentrations of imipramine. Imipramine inhibited A-type delayed rectifier K^+ currents in a concentration-dependent manner. (B) When the inhibitory effect of imipramine was measured, approximately 47 % inhibition was observed at 10 μM (n=5).

vation of the delayed rectifier K^+ currents, 4 sec conditioning steps ranging from -80 to +40 mV, followed by a step to +20 mV, were applied in the control and in the presence of imipramine (Fig. 5A). The measured control currents shown in Fig. 5B and Fig. 5C exhibit the imipramine effect. The data from 4 cells are summarized in Fig. 5D. Imipramine did not change the resulting inactivation-potential relationship for the early peak current. The half-inactivation potential in the control, determined from a Boltzmann function fitted to the data, was 42.5±2.9 mV. In the presence of imipramine, the half-inactivation potential was -45.1±1.7 mV. No significant effect of imipramine was recorded. Experiments to characterize the effects of imipramine on the recovery from the inactivation were also performed. A standard two-pulse protocol was used. Currents were elicited by voltage steps from -80 to 0 mV for 4 sec followed by variable recovery periods at -80 mV and test steps from -80 to 0 mV for 200 ms. The measured control currents are shown in Fig. 6A, and Fig. 6B shows the imipramine effect. The data from 5 cells are summarized in Fig. 6C. The time course of recovery from inactivation occurred with single exponential kinetics. The normalized control peak current recovered with a half-time of 23±2 ms in the control and 24±3 ms in the presence of imipramine. No significant effect of imipramine was recorded.

Effects of imipramine on the K_{ATP} currents

To minimize the activities of voltage-dependent K^+ channels and Ca^{2+}-activated K^+ channels, all currents

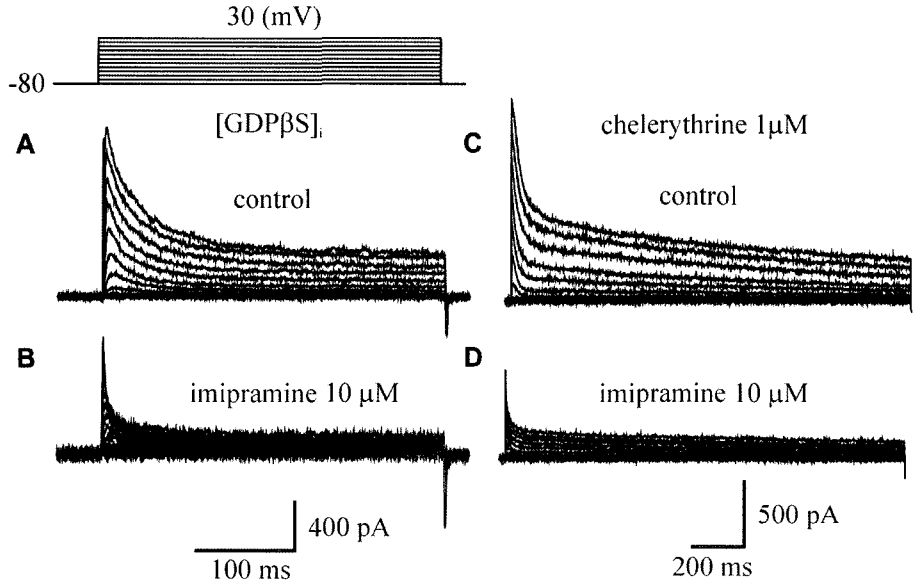

Fig. 4. Effects of intracellular application of GDPβS and protein kinasec inhibitor on the imipramine-induced inhibition of the A-type delayed rectifier K^+ currents. Membrane potential was stepped from -80 mV to test potential between -80 and +30 mV in 10 mV increments. (A) and (C), control. (B), GDPβS (1 mM) did not affect the imipramine (10 μM)-induced inhibition of the A-type delayed rectifier K^+ current (n=3). Chelerythrine did not block the imipramine-induced inhibition (n=4).

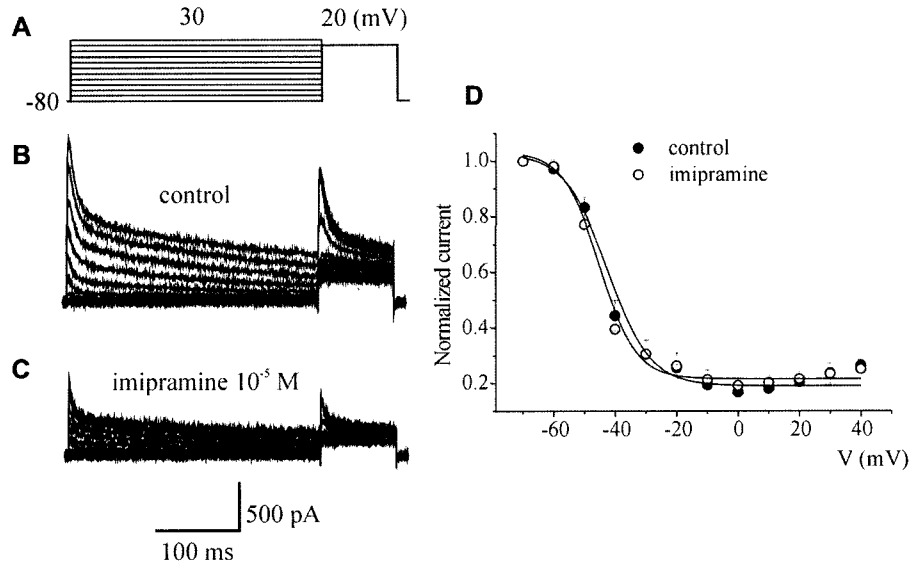

Fig. 5. Effects of imipramine on the voltage-dependent inactivation of the A-type delayed rectifier K^+ currents. Membrane potential was stepped to conditioning potentials between -80 and +30 mV for 4 sec and then stepped to a test potential of +20 mV. (A), protocol. (B), control. (C), in the presence of imipramine (10 μM). (D), the voltage dependence of inactivation of the delayed rectifier K^+ current is shown as a plot of normalized peak outward currents during the test step as a function of the conditioning potential. Data were fitted to the function: I/Imax = 1/11+exp(V-V_{half})/k. Where Imax is the amplitude of the current, V is the value of the test potential, V_{half} is the potential at which 50% of the current was inactivated, and k is the slope of the curves. V_{half} and k were 42.5±2.9 mV and 7.4 in the control, -45.1±1.7 mV and 6.1 in the presence imipramine, respectively (n=4).

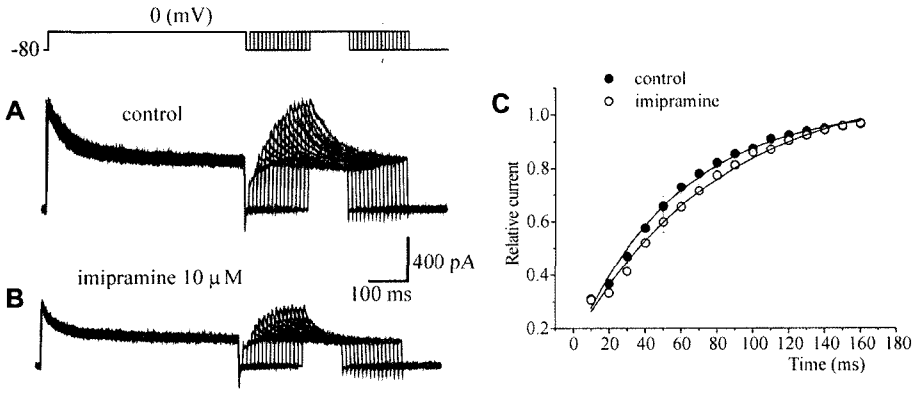

Fig. 6. Effects of imipramine on the recovery from inactivation of A-type delayed rectifier K^+ currents. Membrane potentials were stepped from -80 to 0 mV for 4 sec followed by variable recovery periods at -80 mV and test steps to 0 mV for 200 ms. (A), control. (B), in the presence of imipramine (10 μM). (C), recovery of the delayed rectifier K^+ currents obtained by normalizing the peak current during the test step and plotted as a function of the recovery interval in the control and in the presence of imipramine. Solid lines were fit to the data by single exponential function (n=5).

were recorded at a holding potential of -80 mV and all solutions were buffered to low levels of intracellular Ca^{2+} with 10 mM EGTA. Cells were perfused with external 90 mM K^+ to increase the driving force at this potential. The internal pipette solution contained 140 mM K^+ and 0.1 mM ATP. This protocol has been used for vascular smooth muscle cells (Quayle *et al.*, 1995), gastric smooth muscle cells (Jun *et al.*, 1998) and urinary bladder smooth muscle cells (Bonev and Nelson, 1993).

After the whole-cell configuration was established, inward steady-state currents were induced by increasing the external K^+ concentration from 5 to 90 mM K^+ at a holding potential of -80 mV. The amplitude of the steady-state inward currents was -66±13 pA (n=16). In the presence of external 90 mM K^+, pinacidil (10 μM) activated further inward currents with a mean amplitude of -434±23 (n=11), and glibenclamide (10 μM) suppressed the pinacidil-activated currents by 92±3% (n=5) (Fig. 7A). Imipramine inhibited pinacidil-activated inward currents (Fig. 7B). Imipramine-induced inhibition was not changed by intracellular application of GDPβS (1 μM); there was no difference between control and GDPβS (n=4) (Fig. 8A). To

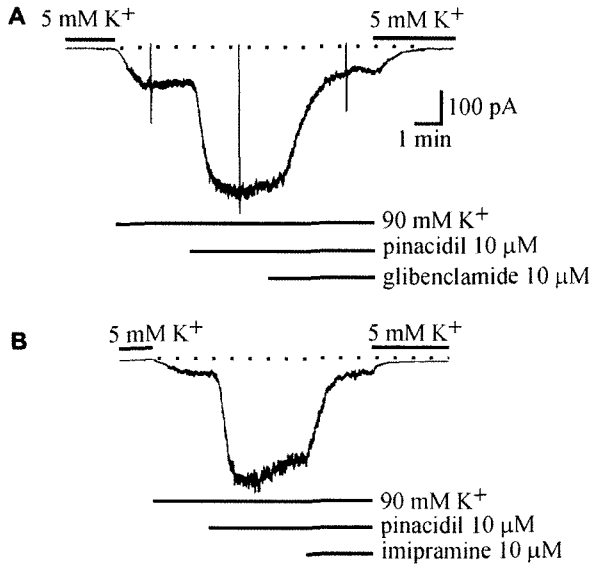

Fig. 7. Effects of imipramine on the recovery from inactivation of K_{ATP} currents. (A), typical trace of glibenclamide-sensitive inward current induced by pinacidil in smooth muscle cells from proximal colon. Current was recorded at a holding potential of -80 mV. The internal pipette solution contained 140 mM K^+, 10 mM EGTA and 0.1 mM ATP. An increase in the external K^+ concentration from 5 to 90 mM induced an inward current. Dotted line indicates the zero current level. In the presence of 90 mM K^+, pinacidil (10 μM) activated further large inward current that was blocked by glibenclamide (10 μM). (B), imipramine inhibited pinacidil-activated currents.

examine the involvement of protein kinase C in the inhibition of K_{ATP} currents by imipramine, we pretreated with protein kinase C inhibitor. Following pretreatment with chelerythrine (1 μM) for 20 min, the inhibition of the pinacidil-activated currents by imipramine was not changed (Fig. 8B). In the presence of chelerythrine, the imipramine-induced inhibition was 90.3±1.8 % (n=4). There was no difference in this inhibition in the absence or presence of the protein kinase C inhibitor (Fig. 8C). Chelerythrine itself had no effects on the pinacidil-activated currents (not shown).

DISCUSSION

In this study, we report that imipramine inhibits A-type delayed rectifier K^+ currents and K_{ATP} currents in a manner independent of G-protein and protein kinase C in murine proximal colonic smooth muscle cells. Imipramine is an antidepressant drug for depression, but has relatively common gastrointestinal adverse motility effects (Gilman *et al.*, 1991). The gastrointestinal motility is ultimately determined by the membrane potential of smooth muscle activity. In a previous study, it was reported that a component of delayed rectifier K^+ current that inactivates relatively rapidly and is sensitive to 4-AP plays an important role in regulating the rhythmic electrical activity of murine proximal colonic smooth muscle cells (Koh *et al.*, 1999).

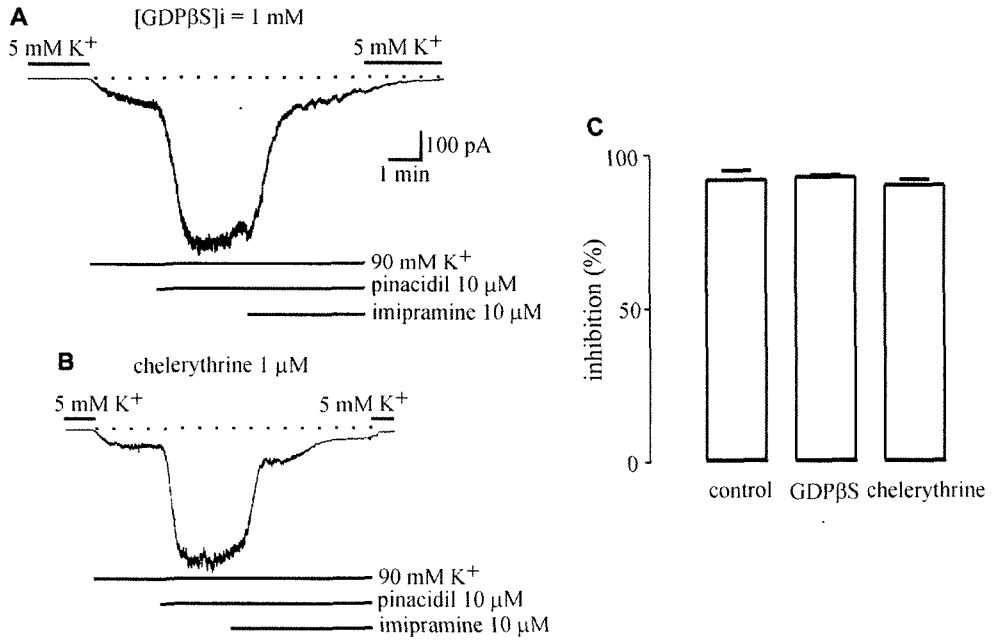

Fig. 8. Effects of intracellular application of GDPβS and protein kinase C inhibitor on the imipramine-induced inhibition of the pinacidil-activated current. The holding potential was -80 mV. (A) and (B), GDPβS (1 μM) and chelerythrine did not affect the imipramine (1 mM)-induced inhibition of the pinacidil-activated current. Dotted line indicates the zero current level. (C), Mean fractional inhibition of the pinacidil-activated currents by imipramine in the absence and presence of intracellular GDPβS and following pretreatment with chelerythrine. The inhibitions by imipramine were 93±0.6% (n=3) and 90.3±1.8% (n=3) in the presence of GDPβS and in the presence of chelerythrine, respectively.

Inhibition of this A-type K^+ current changed the pattern of electrical activity and induced continuous spiking. K_{ATP} channels participate in the regulation of membrane potential and cell excitability in colonic smooth muscles and have a significant open probability under basal conditions (Koh *et al.*, 1998). The inhibition of K_{ATP} channels causes membrane depolarization and leads to an increase in cell excitability, whereas the activation of K_{ATP} channels causes membrane hyperpolarization and leads to a decrease in cell excitability. Thus, the K_{ATP} channels are regulated by dual systems. The activation of protein kinase C inhibits K_{ATP} currents and the activation of protein kinase A activates K_{ATP} currents (Bonev and Nelson, 1996; Kleppisch and Nelson, 1995). Acetylcholine and substance P, which are depolarizing agents in gastrointestinal smooth muscles, are reported to inhibit K_{ATP} channels via G-protein through protein kinase C activation in esophageal (Hatakeyama *et al.*, 1995) and gastric smooth muscle cells (Jun *et al.*, 1998). We have also reported that Phorbol 12, 13-dibutyrate (PDBu), an activator of protein kinase C, inhibited K_{ATP} currents in murine colonic myocytes and that the PDBu effect was blocked by chelerythrine (Jun *et al.*, 2001).

Imipramine inhibited the L-type Ca^{2+} channels in neurons of murine dorsal root ganglion through G- protein activation (Choi *et al.*, 1992) Also, fluoxetine inhibited the delayed rectifier K^+ channel in jejunal smooth muscle cells; the inhibitory effects of fluoxetine were blocked by a protein kinase C inhibitor, indicating that the action of fluoxetine was protein kinase C-dependent (Farrugia, 1996). However, in our experiments, the inhibitory action of imipramine on the A-type delayed rectifier K^+ currents and K_{ATP} currents seems to be due to a direct effect, as the inhibitory action of imipramine on both currents was not blocked by GDPβS. In addition, chelerythrine, a specific protein kinase C inhibitor, did not block the imipramine-induced inhibition. We do not know the reason for the different results between the effect of fluoxetine on the delayed rectifier K^+ channels and the effect of imipramine on the A-type delayed rectifier K^+ channels in murine colonic smooth muscle cells. The difference in K^+ channel type or in the tissues examined may be an explanation for this observed difference. The direct effect of imipramine on the K^+ channels was also reported in neurons (Kuo, 1998) and expressed cells (Sakuta, 1994; Hahn *et al.*, 1999)

In summary, we demonstrated that imipramine inhibits A-type K^+ currents in proximal colonic myocytes and that this action is not mediated through G-protein or protein kinase C. This inhibitory action is a possible mechanism of the imipramine-evoked gastrointestinal side effects.

ACKNOWLEDGEMENTS

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