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Histamine H_1-receptor-mediated modulation of the delayed rectifier K^+ current in guinea-pig atrial cells: opposite effects on I_{Ks} and I_{Kr}

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1 Histamine receptor-mediated modulation of the rapid and slow components of the delayed rectifier K^+ current (I_K) was investigated in enzymatically-dissociated atrial cells of guinea-pigs using the whole cell configuration of the patch clamp technique.

2 Histamine at a concentration of 10 μM enhanced I_K recorded during strong depolarization to potentials ranging from +20 to +40 mV and inhibited I_K recorded during mild depolarization to potentials ranging from −20 to −10 mV. The increase of I_K was more prominent with longer depolarizing pulses, whereas the inhibition of I_K was more marked with shorter depolarizing pulses, suggesting that histamine enhances I_{Ks} (the slow component of I_K) and inhibits I_{Kr} (the rapid component of I_K).

3 The histamine-induced enhancement of I_{Ks} and inhibition of I_{Kr} were abolished by 3 μM chlorpheniramine but not by 10 μM cimetidine, suggesting that these opposite effects of histamine on I_{Kr} and I_{Ks} are mediated by H_1-receptors.

4 In the presence of 5 μM E-4031, an I_{Kr} blocker, histamine hardly affected I_K during mild depolarization although it enhanced I_K during strong depolarization in a concentration-dependent manner. Histamine increased I_{Ks} with EC_{50} value of 0.7 μM. In the presence of 300 μM indapamide, an I_{Ks} blocker, histamine hardly affected I_{Ks} but inhibited I_{Kr} in a concentration-dependent manner. Histamine decreased I_{Kr} with IC_{50} value of 0.3 μM.

5 Pretreatment with 100 nM calphostin C or 30 nM staurosporine, protein kinase C inhibitors, abolished the histamine-induced enhancement of I_{Ks}, but failed to affect the histamine-induced inhibition of I_{Kr}.

6 We conclude that in guinea-pig atrial cells H_1-receptor stimulation enhances I_{Ks} and inhibits I_{Kr} through different intracellular mechanisms.

Keywords: Histamine; H_1 receptor; the delayed rectifier K^+ current; atrial cells; protein kinase C

Abbreviations: APD, action potential duration; ANOVA, analysis of variance; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid; HERG, the human ether-a-go-go related gene; I_K, delayed rectifier K^+ current; $I_{K.ACh}$, muscarinic acetylcholine receptor-operated K^+ current; $I_{K,depo}$, time-dependent current of I_K during depolarizing pulses; I_{Kr}, rapid component of I_K; I_{Ks}, slow component of I_K; $I_{K,tail}$, tail current of I_K; indo-1/AM, the acetoxymethyl ester of indo-1; IP_3, inositol 1,4,5-trisphosphate; KB solution, Kraft-Brühe solution; PKA, protein kinase A; PKC, protein kinase C; TRH, thyrotropin-releasing hormone

Introduction

Tremendous progress has been made in the understanding of the function and diversity of cardiac K^+ channels (Barry & Nerbonne, 1996). The delayed rectifier K^+ current (I_K) is an important contribution to the action potential repolarization in cardiac cells. The K^+ current (I_K) has been characterized in a wide variety of species and tissue types of the heart since the first analysis in sheep Purkinje fibres by Noble & Tsien (1969). It is now well-established that there are two components of I_K that display different time and voltage-dependent properties and pharmacological sensitivities in cardiomyocytes of various animal species including guinea-pigs and humans: rapid (I_{Kr}) and slow components (I_{Ks}) (Sanguinetti & Jurkiewicz, 1990; 1991; Barry & Nerbonne, 1996).

The important K^+ current system has been shown to be regulated by several intracellular mechanisms, such as cyclic AMP-dependent kinase (protein kinase A, PKA) (Walsh & Kass, 1988; Yazawa & Kameyama, 1990) and protein kinase C (PKC) (Tohse *et al*., 1987; Walsh & Kass, 1988). The cyclic AMP-PKA pathway was shown to be involved in β-adrenoceptor-mediated and histamine H_2-receptor-mediated enhancement of I_K (Yazawa & Kameyama, 1990; Yazawa & Abiko, 1993). In addition, Sanguinetti *et al*. (1991) demonstrated that isoproterenol markedly increased the magnitude of I_{Ks} without significant effect on I_{Kr} in guinea-pig ventricular cells. In terms of the enhancement of I_K through the activation of PKC, it was reported that α_1-adrenoceptor stimulation increased the K^+ current in guinea-pig ventricular cells (Tohse *et al*., 1992). However, it has not been determined conclusively which component of I_K is affected by the PKC activation. It is well known that histamine H_1-receptor stimulation is coupled with the phosphoinositide hydrolysis, leading to inositol 1,4,5-trisphosphate (IP_3) production and PKC activation, in a variety of cells (Babe & Serafin, 1995). In guinea-pig atrial cells H_1 receptors are involved in the electromechanical and biochemical responses to histamine (Hattori *et al*., 1988;

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Sakuma *et al*., 1988; Yoshimoto *et al*., 1998) although species and regional differences exist with respect to the receptor subtypes mediating the cardiac response to histamine. Since both I_{Kr} and I_{Ks} are reported to contribute to the action potential repolarization in guinea-pig atrial cells (Sanguinetti & Jurkiewicz, 1991), we thought it would be of interest to examine the effects of H_1 receptor stimulation, associated with PKC activation, on I_K in these cells. In this study we examined the effects of histamine on I_K of guinea-pig atrial cells with special reference to the subtypes of I_K, i.e., I_{Kr} and I_{KS}, by use of patch clamp techniques. The findings presented here indicate that histamine H_1 receptor stimulation enhances I_{Ks} and inhibits I_{Kr}.

Methods

Cell preparations

All experiments were performed under the regulations of the Animal Research Committee of the School of Medicine, Chiba University. Single atrial cells of the guinea-pig heart were isolated by an enzymatic dissociation method, as previously described (Watanabe *et al*., 1996). Guinea-pigs weighing 250–400 g were anaesthetized with pentobarbitone sodium. Their hearts were removed, immediately mounted on a Langendorff apparatus, and retrogradely perfused with (1) normal HEPES-Tyrode solution for 10 min, (2) nominally Ca^{2+}-free Tyrode solution for 10 min, and then (3) Ca^{2+}-free Tyrode solution containing 0.1–0.2 mg/ml collagenase (Wako, Osaka, Japan) for 20–30 min. After digestion, the heart was perfused with a high-K^+ low-Cl^- solution (modified Kraft-Brühe (KB) solution) (Isenberg & Kloeckner, 1982; Sakamoto *et al*., 1998). Atrial tissue was cut into small pieces in the modified KB solution, and the pieces were gently agitated to dissociate cells. The cell suspension was stored in a refrigerator (4°C) and used on the same day.

Solutions

The composition of the normal HEPES-Tyrode solution was (mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5 and HEPES-NaOH buffer 5 (pH 7.4). The nominally Ca^{2+}-free Tyrode solution used for the cell isolation procedure was prepared by simply omitting $CaCl_2$, from the normal Tyrode solution. The composition of the modified KB solution was (mM): KOH 70, L-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, EGTA 1, and HEPES-KOH buffer 10 (pH 7.4). The external solution for the measurement of I_K was normal Tyrode solution plus 1 μM nifedipine. The composition of the pipette solution was (mM): potassium aspartate 110, KCl 20, $MgCl_2$ 1, potassium ATP 5, potassium phosphocreatine 5, EGTA 10, and HEPES-KOH buffer 5 (pH 7.4). The free Ca^{2+} concentration in the pipette solution was adjusted to pCa 8 by adding $CaCl_2$ according to the calculation by Fabiato & Fabiato (1979) with the correction of Tsien & Rink (1980). In part of experiments GTP (100 μM) was added to the pipette solution.

Whole-cell current recordings

Whole-cell membrane currents were recorded by the patch-clamp method (Hamill *et al*., 1981). Single atrial cells were placed in a recording chamber (1 ml volume) attached to an inverted microscope (model IMT-2, Olympus, Tokyo, Japan) and superfused with the HEPES-Tyrode solution at a rate of 3 ml min^{-1}. The temperature of the external solution was kept constant at 36.0±1.0°C. Patch pipettes were made from glass capillaries with a diameter of 1.5 mm using a vertical microelectrode puller (model PB-7, Narishige, Tokyo, Japan). They were filled with an internal solution, and their resistance was 2–4 MΩ. After the gigaohm seal between tip and cell membrane was established, the membrane patch was disrupted by more negative pressure to make the whole-cell voltage-clamp mode. The electrode was connected to a patch-clamp amplifier (model CEZ-2300, Nihon Koden, Tokyo, Japan). Recording signals were filtered at 1 kHz bandwidth, and series resistance was compensated by 40–70%. Command pulse signals were generated by a 12-bit digital-to-analogue converter controlled by pCLAMP software (Axon Instrument, Inc., Foster City, CA, U.S.A.). Current signals were digitized at 2 kHz and stored on the hard disc of an IBM-compatible computer (Compaq Prolinea 4/50 with a 1 Gbyte hard disc, Houston, TX, U.S.A.). A liquid junction potential between the internal solution and the bath solution of −8 mV was corrected.

Membrane currents were recorded by delivering 300 ms depolarizing pulses from a holding potential of −40 mV at a rate of 0.1 Hz and effects of histamine on the membrane currents were examined. The delayed rectifier K^+ current (I_K) was elicited by delivering the depolarizing pulses from a holding potential of −40 mV after the inhibition of the L-type Ca^{2+} current by nifedipine. The amplitude of the deactivating current ($I_{K,tail}$) was measured as the difference between the holding current and the peak current that was actually recorded upon the clamp back to the holding potential. The amplitude of the time-dependent current activated during depolarizing pulses ($I_{K,depo}$) was also measured. The I_K of guinea-pig atrial cells consists of two components, rapidly activating component (I_{Kr}) and slowly activating component (I_{Ks}) (Sanguinetti & Jurkiewicz, 1991). In order to determine whether histamine affects I_{Kr} and/or I_{Ks}, effects of histamine on the I_K elicited by short depolarizing pulses (200 ms) and long depolarizing pulses (3 s) were evaluated. In addition, effects of histamine on I_{Ks} were examined in the presence of the I_{Kr} blocker E-4031 (5 μM), and those on I_{Kr} were examined in the presence of the I_{Ks} blocker indapamide (300 μM). In preliminary experiments we confirmed that E-4031 at 5 μM can produce a full inhibition of I_{Kr}. We used 300 μM of indapamide because the drug at the concentration reportedly suppressed I_{Ks} with little influence on I_{Kr} (Turgeon *et al*., 1994). In a part of experiments, influences of chlorpheniramine, a H_1 antagonist, or cimetidine, a H_2 antagonist, on the histamine-induced modulation of I_K were evaluated to determine the receptor subtype involved. Effects of PKC inhibitors, staurosporine and calphostin C, on the histamine-induced modulation of I_K were also evaluated.

Drugs

Drugs used in this study were as follows: histamine dihydrochloride, cimetidine, dl-chlorpheniramine maleate, calphostin C, staurosporine (Wako, Osaka, Japan), nifedipine, indapamide (Sigma, U.S.A.), E-4031 (N-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl] methanesulphonamide dihydrochloride dihydrate] (Eisai Co., Tokyo, Japan). Indapamide stock solutions (100 mM) were prepared in 0.5 M KOH and diluted with the external solution from which KCl was omitted from the normal HEPES-Tyrode solution. Then, we finally adjusted K^+ concentration to 5.4 mM and the pH of the solution to 7.40 with 1 M KCl and 1 M KOH. Nifedipine was dissolved in ethanol and the final

concentration of ethanol was less than 0.1%. Calphostin C and staurosporine were dissolved in dimethylsulphoxide and the final concentration of dimethylsulphoxide was less than 0.1%. It was confirmed that neither ethanol nor dimethylsulphoxide at the concentrations used showed any appreciable influence on the membrane current. The other compounds were dissolved in distilled water.

Statistics

All values are presented mean ± s.e.mean. Student's *t*-test and ANOVA were used for statistical analyses. *P* value of less than 0.05 was considered significant. The concentration-effect data were fitted and the EC_{50} values or the IC_{50} values were obtained using Delta Graph Professional (Delta Point, Polaroid computing, Tokyo, Japan).

Results

Modulation of the delayed rectifier K^+ current by histamine

Effects of histamine on the membrane current system were examined in guinea-pig atrial cells. Membrane currents were elicited by 300 ms test pulses to various potentials from a holding potential of −40 mV at 0.1 Hz after the blockade of L-type Ca^{2+} current by 1 μM nifedipine. Representative changes in the membrane currents after 10 μM histamine are shown in Figure 1A, and the data of the current-voltage relations for the current measured after repolarization to −40 mV from the indicated test potential ($I_{K,tail}$) are summarized in Figure 1B. This concentration of histamine was reported to produce the maximal positive inotropic response in guinea-pig atrial preparations (Sakuma *et al*., 1988) and the maximal increase in the Ca^{2+} transient in guinea-pig atrial cells (Yoshimoto *et al*., 1998). Histamine enhanced the late outward current elicited by depolarizing test pulses ($I_{K,depo}$) to voltage range from +10 to +40 mV concomitantly with the increase in the $I_{K,tail}$ elicited by the clamp back to the holding potential of −40 mV. On the other hand, histamine inhibited the $I_{K,depo}$ elicited by depolarizing test pulses from −30 to −10 mV with the inhibition of the $I_{K,tail}$. The enhancement of I_K at +40 mV and the inhibition of I_K at −10 mV were 96 ± 15% ($P<0.05$) and −47 ± 12% ($P<0.05$), respectively. These effects were partially reversed upon washout of histamine. These findings indicate that histamine enhances I_K during strong depolarization and inhibits I_K during mild depolarization, suggesting the opposite effects of histamine on two components of I_K in guinea-pig atrial cells.

In order to test whether the histamine-induced modulation of I_K is mediated by H_1-receptors or H_2-receptors, we investigated the effects of histamine on I_K in the presence of the H_1-antagonist chlorpheniramine or the H_2-antagonist cimetidine. In the presence of 3 μM chlorpheniramine, both the enhancement of I_K during strong depolarization and the inhibition of I_K during mild depolarization after 10 μM histamine were abolished, as shown in Figure 2A,B. However, cimetidine at a concentration of 10 μM failed to affect the histamine-induced enhancement or inhibition of I_K, as shown in Figure 2C,D. These results suggest that the histamine-induced modulation of I_K is mediated by H_1-receptors.

In a part of experiments, we evaluated histamine-induced modulation of I_K using a GTP(100 μM)-containing pipette solution. Histamine at a concentration of 10 μM similarly enhanced I_K during strong depolarization and inhibited I_K during mild depolarization. The enhancement of I_K at

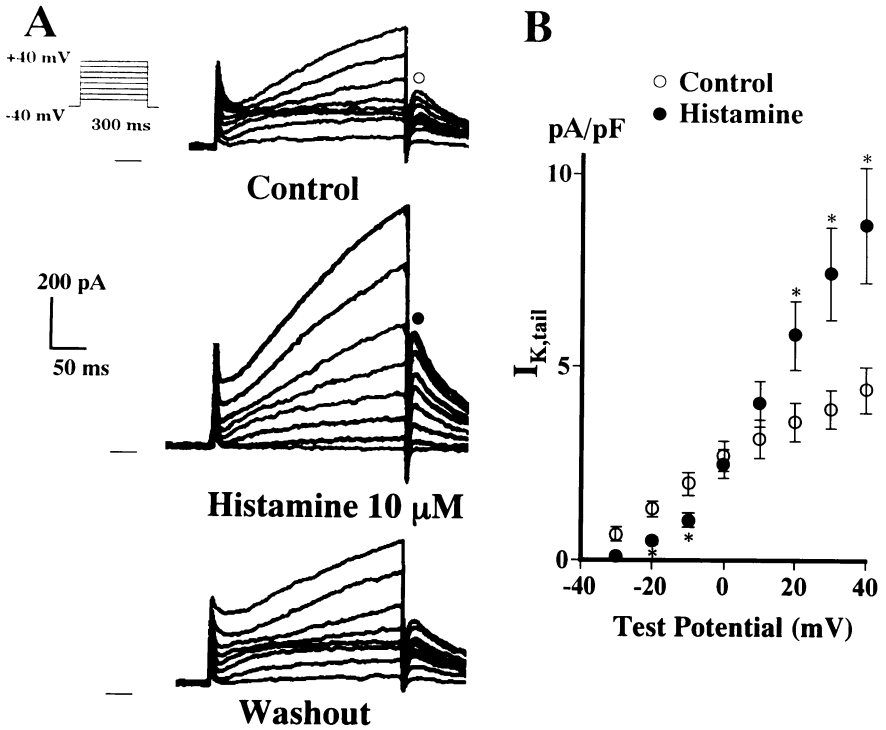

Figure 1 Effects of histamine on membrane currents in a guinea-pig atrial cell. (A) Actual current traces elicited by 300 ms depolarizing pulses from a holding potential of −40 mV before (upper), during exposure to 10 μM histamine (middle) and after washout of histamine (lower). The external solution contained 1 μM nifedipine. (B) Summarized data of current-voltage relations for the current measured after repolarization to −40 mV from the indicated test potential ($I_{K,tail}$) before and during exposure to histamine. Data represent mean ± s.e.mean of five cells.*$P<0.05$ vs control.

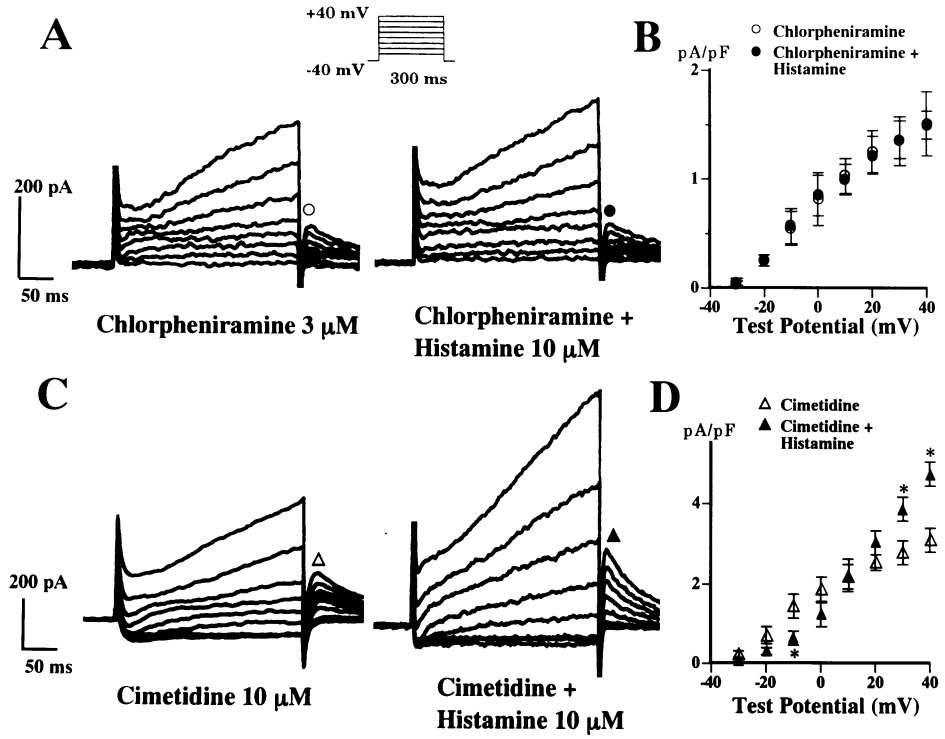

Figure 2 Effects of histamine on the delayed rectifier K^+ current (I_K) in the presence of H_1- or H_2-antagonist. (A) Current traces elicited by 300 ms depolarizing pulses from a holding potential of −40 mV in control condition with 3 μM chlorpheniramine (left) and after the addition of 10 μM histamine (right). (B) Graph showing I_K measured after clamp back to −40 mV from the indicated test potential ($I_{K,tail}$), obtained from A. Data represent mean±s.e.mean of five cells. (C) Current traces elicited by 300 ms depolarizing pulses from a holding potential of −40 mV in control condition with 10 μM cimetidine (left) and after the addition of 10 μM histamine (right). (D) Graph showing I_K measured after clamp back to −40 mV from the indicated test potential ($I_{K,tail}$), obtained from C. Data represent mean±s.e.mean of five cells. *$P<0.05$ vs control.

+40 mV and the inhibition of I_K at −10 mV were 86±24% ($P<0.05$) and −52±7% ($P<0.05$), respectively. Neither the magnitude of the inhibition nor that of the enhancement was significantly different from that observed using a pipette solution without GTP.

It is known that I_{Kr} is activated rapidly with mild depolarization whereas I_{Ks} is activated slowly with a sigmoidal time course at more positive potentials (Sanguinetti & Jurkiewicz, 1991). To test whether histamine differentially affects I_K, the following experiments were conducted. Short (200 ms) or long (3 s) depolarizing pulses were applied from a holding potential of −40 mV to various potentials at a rate of 0.1 Hz. Histamine-induced enhancement of I_K during depolarizing pulses ($I_{K,depo}$) and I_K after repolarization to a holding potential from test pulses ($I_{K,\ tail}$) was somewhat prominent with long pulses, compared to short pulses. In addition, it was more marked during strong depolarization to potentials ranging from +20 to +60 mV than during mild depolarization to potentials ranging from −10 to +10 mV (Figure 3). In contrast, the inhibitory effect of histamine on $I_{K,depo}$ and $I_{K,tail}$ was more prominent with short pulses, compared to long pulse, and was more marked during mild depolarization to potentials ranging from −10 to +10 mV than during strong depolarization to potential from +20 to +60 mV. These results also suggest that histamine enhances I_{Ks} and inhibits I_{Kr}.

To confirm the histamine-induced enhancement of I_{Ks} and inhibition of I_{Kr}, we investigated the effect of histamine on I_K in the presence of I_{Kr} or I_{Ks} blocker. After the full inhibition of I_{Kr} by 5 μM E-4031, histamine at a concentration of 10 μM hardly affected I_K after the clamp back to the holding current ($I_{K,tail}$) during mild depolarization to potentials ranging from −30 to 0 mV, whereas it markedly enhanced I_K during strong depolarization to potentials ranging from +10 to +40 mV (Figure 4A,B). The histamine-sensitive tail current in the presence of 5 μM E-4031 is shown in Figure 4C. The histamine-induced I_K was activated at potentials positive to −10 mV and the amplitude of I_K increased as the magnitude of depolarization increased. These results indicate that histamine enhances I_{Ks} alone in the presence of E-4031. Histamine at a concentration of 10 μM increased I_K at +40 mV by 161±39%. Histamine increased I_{Ks} in a concentration-dependent manner, and the EC_{50} value of histamine for increasing $I_{K,tail}$ at +40 mV was 0.7 μM (Figure 4D).

Recently indapamide, a diuretic agent, was reported to produce a selective inhibition of I_{Ks} (Turgeon *et al.*, 1994). We investigated the effect of histamine on I_{Kr} in the presence of 300 μM indapamide. After the inhibition of I_{Ks} by 300 μM indapamide, histamine at a concentration of 10 μM hardly affected I_{Ks}, but inhibited I_{Kr} significantly, as shown in Figure 5A,B. The histamine-sensitive tail current in the presence of indapamide is shown in Figure 5C. The histamine-sensitive I_K was activated at potentials positive to −30 mV and peaked around 0 mV. In addition, a marked inward rectification was observed at potentials positive to 0 mV. These findings indicate that histamine-sensitive current in the presence of indapamide would be I_{Kr}, although the histamine-induced enhancement of I_{Ks} at very positive potential could not be completely abolished. Histamine inhibited I_{Kr} at −10 mV by 35±4% in the presence of indapamide. Histamine inhibited I_{Kr} in a concentration-dependent manner, and the IC_{50} value of

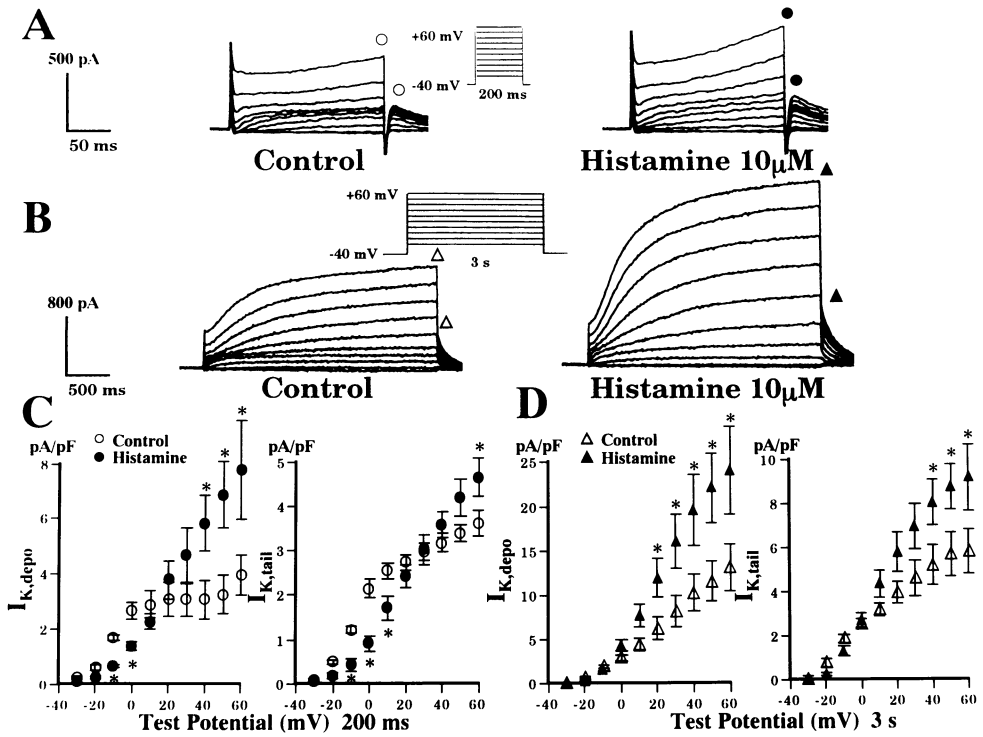

Figure 3 Effects of histamine on the delayed rectifier K^+ current (I_K) elicited by short and long test pulses. (A) Current traces recorded during 200 ms depolarizing pulses from a holding potential of −40 mV before (left) and after exposure to 10 μM histamine (right) in a single atrial cell. (B) Current traces recorded during 3 s depolarizing pulses from a holding potential of −40 mV before (left) and after exposure to 10 μM histamine (right) in a single cell. (C) Graphs showing I_K measured at the end of 200 ms test pulses to the indicated test potential ($I_{K,depo}$, left) and that measured after repolarization to −40 mV from the indicated test potential ($I_{K,tail}$, right). Data represent mean±s.e.mean of five cells. $^*P<0.05$ vs control. (D) Graphs showing I_K measured at the end of 3 s test pulses to the indicated test potential ($I_{K,depo}$, left) and that measured after repolarization to −40 mV from the indicated test potential ($I_{K,tail}$, right). Data represent mean±s.e.mean of five cells. $^*P<0.05$ vs control.

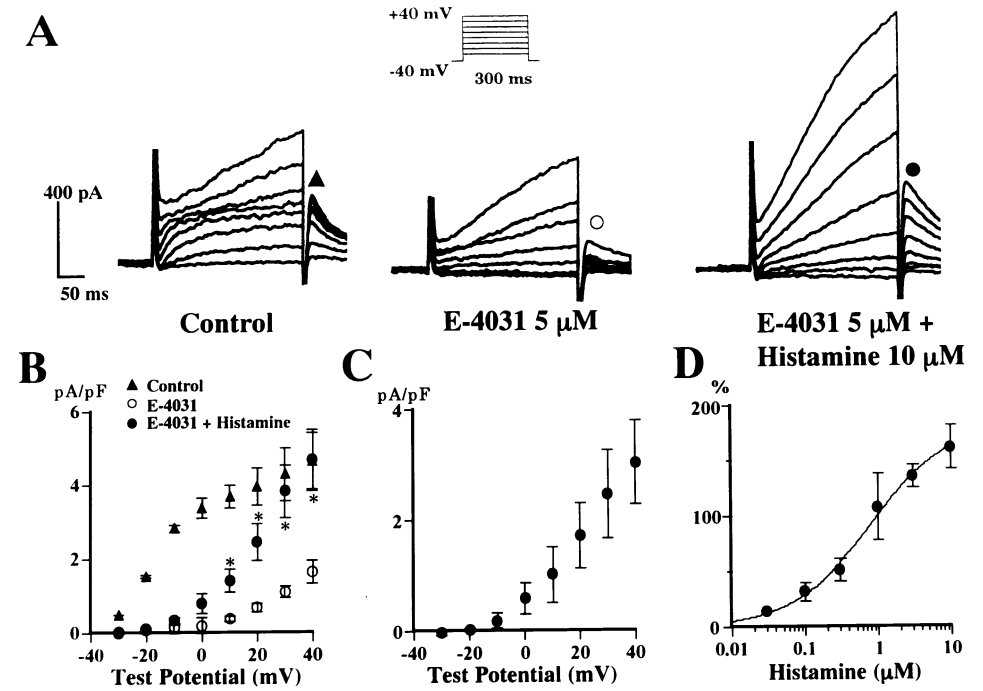

Figure 4 Effects of histamine (10 μM) on the delayed rectifier K^+ current (I_K) in the presence of the I_{Kr} blocker E-4031. (A) Current traces elicited by 300 ms depolarizing pulses from a holding potential of −40 mV in the control condition (left), in the presence of 5 μM E-4031 (middle) and after the addition of 10 μM histamine (right). (B) Graphs showing I_K measured after clamp back to −40 mV from the indicated test potential ($I_{K,tail}$) are shown. Data represent mean±s.e.mean of five cells. $^*P<0.05$ vs E-4031 alone. (C) Current-voltage relation of histamine-sensitive tail currents in the presence of E-4031. Data represent mean±s.e.mean of five cells. (D) Concentration-response curve for the increasing effect of histamine on I_K are shown. Per cent increases in $I_{K,tail}$ are indicated on the ordinate and the concentrations of histamine are on the abscissa. Value are expressed as mean±s.e.mean of 4–6 experiments.

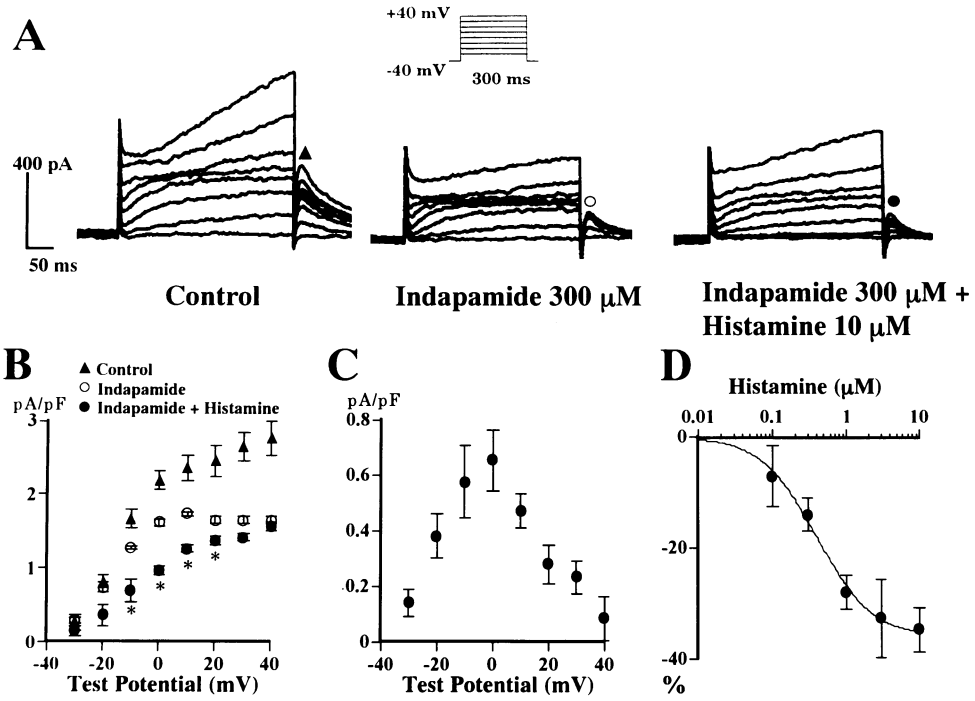

Figure 5 Effect of histamine (10 μM) on the delayed rectifier K^+ current (I_K) in the presence of the I_{Ks} blocker indapamide. (A) Current traces elicited by 300 ms depolarizing pulses from a holding potential of -40 mV in the control condition (left), in the presence of 300 μM indapamide (middle) and after the addition of 10 μM histamine (right). (B) Graphs showing I_K measured after clamp back to -40 mV from the indicated test potential ($I_{K,tail}$) are shown. Data represent mean$\pm$s.e.mean of five cells. *$P<0.05$ vs indapamide alone. (C) Current-voltage relation of histamine-sensitive tail currents in the presence of indapamide. Data represent mean$\pm$s.e.mean of five cells. (D) Concentration-response curve for the inhibitory effect of histamine on I_K are shown. Per cent inhibition of $I_{K,tail}$ is indicated on the ordinate and the concentrations of histamine are on the abscissa. Value are expressed as mean$\pm$s.e.mean of 4–6 experiments.

histamine for inhibiting $I_{K,tail}$ at -10 mV was 0.3 μM (Figure 5D).

Intracellular mechanisms of histamine-induced modulation of I_K

It is well-established that H_1 receptor stimulation can increase phosphoinositide hydrolysis and then activate PKC in cardiac tissues of various species (Sakuma *et al*., 1988; Hattori *et al*., 1990). We investigated the influences of PKC inhibitors on the histamine-induced modulation of I_K. The histamine-induced increases of I_{Ks} was abolished by 100 nM calphostin C, a PKC inhibitor (Figure 6A,B). However, the inhibition of I_{Kr} by histamine could be still observed in the presence of calphostin C. The histamine-sensitive I_K in the presence of calphostin C was activated at potentials positive to -30 mV and showed a marked inward rectification (Figure 6C).

Summarized data of influences of calphostin C on the histamine-induced modulation of I_{Kr} and I_{Ks} are shown in Figure 6D. In the absence of any PKC inhibitor, histamine at a concentration of 10 μM decreased $I_{K,tail}$ at -10 mV by $43\pm10\%$ and increased $I_{K,tail}$ at $+40$ mV by $96\pm19\%$. However, in the presence of 100 nM calphostin C the histamine-induced increase of $I_{K,tail}$ at $+40$ mV was significantly reduced to $6\pm3\%$ although the histamine-induced decrease of $I_{K,tail}$ at -10 mV ($45\pm13\%$) was comparable to that observed in the control condition. In addition, the histamine-induced increase of I_{Ks} but not decrease of I_{Kr} was abolished by staurosporine, another PKC inhibitor. In the presence of 30 nM staurosporine, the histamine-induced increase of $I_{K,tail}$ at $+40$ mV was $5\pm3\%$ ($P<0.01$ vs control condition) while the decrease of $I_{K,tail}$ at -10 mV was $29\pm2\%$ (n.s. vs control condition) in five cells. These results suggest that the enhancement of I_{Ks} is mainly mediated by protein kinase C activation whereas mechanism(s) other than PKC activation appear to be involved in the I_{Kr} inhibition.

Discussion

Histamine produces cardiac actions by interacting directly with specific receptors in various animal species. However, there are species and regional differences with respect to the subtypes of histamine receptors mediating the cardiac responses. In guinea-pig atrial muscles histamine produces a positive inotropic response through the activation of H_1 receptors (Reinhardt *et al*., 1974; Steinberg & Holland, 1975), whereas in guinea-pig ventricular myocardium the positive inotropic effect is mediated by H_2 receptors (Verma & McNeill, 1977; Hattori *et al*., 1994). It is generally accepted that H_1-receptor activation leads to the acceleration of phosphoinositide hydrolysis with resultant production of inositol 1,4,5-trisphosphate and diacylglycerol (Babe & Serafin, 1995). Since I_K of atrial cells is composed of two distinct components, I_{Kr} and I_{Ks} (Sanguinetti & Jurkiewicz, 1991), we thought it would be of interest to examine the effect of histamine on I_K in these cells.

It has long been known that catecholamines enhance I_K and shorten cardiac action potential (Carmeliet & Vereecke, 1969; Tsien *et al*., 1972; Kass & Wiegers, 1982). The β-adrenoceptor-mediated enhancement of I_K has been ascribed to the activation of cyclic AMP-PKA pathway (Yazawa & Kameyama, 1990). Sanguinetti *et al*. (1991) have demonstrated that the β-agonist isoproterenol markedly increased the magnitude of I_{Ks} without significant effect on I_{Kr} in guinea-pig ventricular myocytes. Therefore, it has been postulated that the I_K regulated by PKA would be I_{Ks} rather than I_{Kr}. It has been

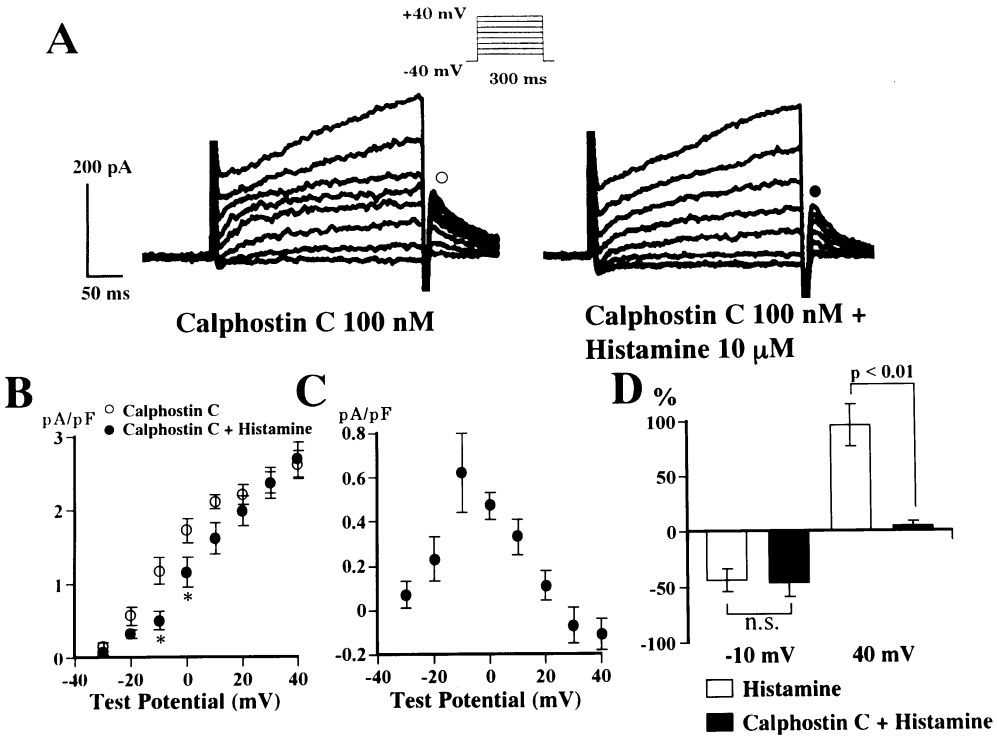

Figure 6 Influence of protein kinase C inhibitor on the histamine-induced modulation of the delayed rectifier K^+ current (I_K). (A) Current traces elicited by 300 ms depolarizing pulses from a holding potential of −40 mV in control condition with 100 nM calphostin C (left) and after the addition of 10 μM histamine (right). (B) Graph showing I_K measured after clamp back to −40 mV from the indicated test potential ($I_{K,tail}$). Data represent mean±s.e.mean of five cells. $^*P<0.05$ vs control. (C) Current-voltage relation of histamine-sensitive tail currents in the presence of calphostin C. Data represent mean±s.e.mean of five cells. (D) Summarized data of influences of calphostin C on the histamine-induced modulation of I_K. Per cent changes in $I_{K,tail}$ after 10 μM histamine at −10 mV and +40 mV in the absence and presence of 100 nM calphostin C are shown. Note that calphostin C abolished the histamine-evoked I_{Ks} enhancement but not I_{Kr} inhibition.

also reported that histamine H_2 receptor stimulation increased I_K by cyclic AMP-dependent phosphorylation in guinea-pig ventricular cells (Hescheler *et al*., 1987; Yazawa & Abiko, 1993). In terms of PKC-mediated regulation of I_K, phorbol esters were shown to increase I_K in guinea-pig ventricular cells (Tohse *et al*., 1987; Walsh & Kass, 1988). In addition, α_1-adrenoceptor stimulation has been demonstrated to enhance I_K through the activation of PKC in guinea-pig ventricular cells (Tohse *et al*., 1992). The I_K increased by phorbol esters and α-agonist in these studies was evoked by long depolarizing pulses (1.5–3 s) in a K^+-free external solution, and the increase of I_K was more marked during strong depolarization pulses to around +50 mV than during mild depolarization to about 0 mV. A decrease in extracellular K^+ concentration was reported to potentiate the inward rectification of I_{Kr}, thereby suppress the outward I_{Kr} current (Yang *et al*., 1997). Therefore the I_K increased by phorbol esters and α-agonist would be expected to be I_{Ks} rather than I_{Kr}. In the present study histamine H_1-receptor stimulation increased I_K even in the presence of the I_{Kr} blocker E-4031 and the enhancement of I_K was completely abolished by the PKC inhibitors such as calphostin C and staurosporine. Accordingly, it can be concluded that I_{Ks} can be enhanced by the activation of H_1-receptor-PKC pathway. Recent studies have revealed that KvLQT1 and minK (IsK) coassemble to form the channel underlying I_{Ks} (Barhanin *et al*., 1996; Sanguinetti *et al*., 1996). It was previously reported that the current that was expressed in *Xenopus* oocytes by injecting mRNA of minK was enhanced by phorbol ester and cyclic AMP analogue (Varnum *et al*., 1993). Recently it has been also shown that activation of P_2-purinoceptors by extracellular ATP selectively enhances I_{Ks} through intracellular mechanisms independent of protein kinase A or protein kinase C in guinea-pig atrial myocytes (Matsuura *et al*., 1996; Matsuura & Ehara, 1997). Thus, I_{Ks} may be enhanced not only by activation of PKA and PKC but also *via* other mechanism(s).

I_{Kr} is known to be specifically blocked by methanesulfonanilide class III antiarrhythmic drugs such as E-4031, sotalol and dofetilide (Sanguinetti & Jurkiewicz, 1990; Carmeliet, 1992). Recent studies have shown that the HERG gene encodes the I_{Kr} channels and mutations of HERG cause long QT syndrome, an inherited abnormality of cardiac repolarization that is associated with an increased risk of polymorphic ventricular arrhythmias called torsades de pointes (Curran *et al*., 1995; Trudeau *et al*., 1995; Sanguinetti *et al*., 1995). To our best knowledge, however, receptor-mediated regulation of I_{Kr} has not been reported in native cardiac myocytes. The present study has shown for the first time that histamine H_1-receptor stimulation inhibits I_{Kr} in guinea-pig atrial cells. Although the histamine-induced enhancement of I_{Ks} was readily abolished by either calphostin C or staurosporine, these PKC inhibitors failed to affect the histamine-induced inhibition of I_{Kr}. More recently, however, Barros *et al*. (1998) have demonstrated that thyrotropin-releasing hormone (TRH) receptor activation inhibited the HERG channel current in *Xenopus* oocytes co-expressing the channel and receptor proteins and a phorbol ester mimicked the K^+ channel inhibition, suggesting the involvement of PKC. The discrepancy between our and their studies might stem from the differences of the receptor systems (H_1 receptor and TRH receptor) and/or the

materials (native atrial myocyte and *Xenopus* oocyte expression system) studied. Whatever the mechanism(s) involved, H_1 receptor stimulation can inhibit I_{Kr}.

It is known that HERG messages are abundantly expressed not only in the heart but also in the brain (Wymore *et al*., 1996). Although the role of HERG channels in neuronal function is not completely understood, they have been implicated in the control of the resting membrane potential associated with the cell cycle, the neuritogenesis, the differentiation of neuronal cells and the neuronal spike-frequency adaptation (Arcangeli *et al*., 1993; 1995; Faravelli *et al*., 1996; Chiesa *et al*., 1997). Since histaminergic system including H_1-receptors is widely distributed in the brain (Bloom, 1995), the H_1-receptor-mediated inhibition of the HERG channel may play an important role in the central nervous system.

Histamine H_1-receptor stimulation was shown to prolong APD in guinea-pig atrial cells (Hattori *et al*., 1988; Yoshimoto *et al*., 1998). The ionic mechanism(s) of the H_1-receptor-mediated action potential prolongation have not been fully understood. Yoshimoto *et al*. (1998) failed to detect an increase in the L-type Ca^{2+} current during H_1-receptor stimulation although an increase in the $[Ca^{2+}]_i$ transient was observed in indo-1/AM loaded atrial myocytes of guinea-pigs. They ascribed the APD prolongation to the inhibition of the background muscarinic K^+ current ($I_{K.ACh}$) because H_1-receptor stimulation was reported to inhibit the carbachol-induced $I_{K.ACh}$ in guinea-pig atrial myocytes (Tohse *et al*., 1995). In the present study histamine enhanced I_{Ks} and inhibited I_{Kr}. The opposite effects of histamine on two components of I_K might cancel out. However, the APD of atrial cells is shorter than that of ventricular cells. In addition, the current density of I_{Kr} in atrial cells was reported to be 2.5 times higher than that measured in ventricular cells of guinea-pigs (Sanguinetti & Jurkiewicz, 1991). Therefore, the I_{Kr} inhibition may in part contribute to the H_1-receptor-mediated APD prolongation in atrial cells at slow heart rate, where I_{Kr} plays a more important role in the action potential repolarization than I_{Ks}.

In conclusion, histamine H_1 receptor stimulation enhances I_{Ks} and inhibits I_{Kr} through different intracellular mechanisms in cardiomyocytes.

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