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Modulation by histamine of the delayed outward potassium current in guinea-pig ventricular myocytes

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1 Histamine receptor-mediated modulation of the delayed outward potassium current (I_K) was investigated in guinea-pig single ventricular cells by the whole-cell voltage clamp.

2 Histamine increased I_K in a dose- dependent manner with a half-maximum dose of 3.8×10^{-8} M. Histamine (10^{-6} M) increased I_K by a factor of 3.02 without a significant change in the current kinetics. The threshold dose of histamine for increasing I_K was 10^{-9} M and this value was similar to that for calcium current.

3 Cimetidine decreased I_K in the presence of histamine, by shifting the dose-response curve to histamine to the right. The pA_2 value of cimetidine against histamine was 6.38.

4 Forskolin did not increase I_K after application of 10^{-6} M histamine, and histamine scarcely increased I_K in the presence of a heat-stable inhibitor of cyclic AMP-dependent protein kinase (PKI).

5 We conclude that stimulation by histamine of I_K is mainly by way of the H_2-receptor, and is mediated by cyclic AMP-dependent phosphorylation.

Keywords: Potassium channels; histamine; cimetidine; chlorpheniramine; heart ventricle

Introduction

The delayed outward K^+ current (I_K) is activated during the plateau phase of the action potential and contributes to its duration. It is known that histamine increases I_K in ventricular cells (Hescheler *et al.*, 1987). It has been hypothesized that an underlying mechanism for this effect is phosphorylation of the channel by adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-dependent protein kinase (PKA), by analogy with stimulation by histamine of Ca^{2+} current (I_{Ca}) in cardiac ventricular cells (Hescheler *et al.*, 1987).

It is also known that an agonist at the β-adrenoceptor increases I_K and I_{Ca} in ventricular cells. It has been hypothesized that this effect is mediated by phosphorylation of the channel by PKA. The threshold dose of isoprenaline for increasing I_K is near 10^{-9} M (Yazawa & Kameyama, 1990), and this value is similar to that for I_{Ca} (Kameyama *et al.*, 1985). Moreover, the maximum concentrations for increasing the two different types of current are nearly the same. From these results, we can hypothesize that the activation of the common phosphorylation pathway modulates both I_K and I_{Ca} at a physiological temperature.

However, it has been recently suggested that I_K and I_{Ca} may possess different sensitivities to protein phosphorylation mediated by cyclic AMP even in the same cell (Tanaka *et al.*, 1991). It has also been reported that I_K is under control of protein kinase C (Tohse *et al.*, 1987; Walsh & Kass, 1988). It is also possible that another cascade might participate in stimulation by histamine in addition to the cyclic AMP pathway.

To address these questions, we investigated the intracellular mechanism underlying stimulation by histamine of I_K in single ventricular cells by using the whole-cell patch clamp method.

Methods

Cell preparation

Single ventricular cells were obtained from the adult guinea-pig heart by an enzymatic dissociation method (Yazawa *et al.*, 1990). In brief, a dissected heart was mounted on a Langendorff apparatus and perfused with nominally Ca^{2+}-free Tyrode solution containing collagenase (Yakult, Japan; 0.16 mg ml^{-1}) at 37°C. After 8–15 min of the collagenase treatment, the enzyme solution was washed out with a storage solution (see below). The ventricle was cut into pieces, and then the dispersed cells were filtered through 210 μm mesh and kept in the storage solution. The cells were subsequently incubated with the storage solution containing both protease 0.04 mg ml^{-1} (Nagase, Japan, Alkaline protease) and deoxyribonuclease, 0.02 mg ml^{-1} (Tokyo Kasei, Japan) for 10–15 min. The cells were washed twice by centrifugation and stored at 4°C in the storage solution.

Solutions

The composition of the external solutions was as follows. Normal Tyrode solution (mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.25, HEPES 5, glucose 5.6, pH to 7.4 with NaOH. The storage solution contained (mM): KOH 70, KCl 40, L-glutamic acid 50, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, HEPES 10, EGTA 0.5, pH to 7.4 with KOH. The Na^+-, K^+ and/or Ca^{2+}-free solution (test solution) contained (mM): N-methyl-D-glucamine 149, $MgCl_2$ 5, HEPES 5, nisoldipine 0.003, pH to 7.4 with HCl; and/or $CaCl_2$ 1.8. The pipette solution contained (mM): KOH 110, KCl 20, $MgCl_2$ 1, K_2ATP 5, potassium creatine phosphate 5, HEPES 5, pH to 7.4 with aspartic acid. Thus the total K^+ concentration in the pipette solution was 150 mM. The concentration of Ca^{2+} in the internal solution was pCa 8 (10 mM EGTA + 1.43 mM Ca^{2+}), calculated by Fabiato & Fabiato's (1979) equations with the correction by Tsien & Rink (1980). The external solutions were warmed by a water jacket before entering the recording chamber. All the experiments were performed at 35–37°C.

Drugs

The following drugs were used: histamine (Nakarai, Japan), forskolin, chlorpheniramine (both from Research Biochemicals Inc. U.S.A.), cimetidine (Funakoshi, Japan) and a heat-stable inhibitor of cyclic AMP-dependent protein kinase (PKI_{5-24}) (American Peptide Company Inc. U.S.A.). Nisoldipine (generous gift from the Bayer Company, Germany)

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was dissolved in dimethyl sulphoxide as a 10 mM stock solution.

Recording of the delayed outward potassium current (I_K)

The delayed outward potassium current (I_K) was recorded in Na^+-, K^+-, 3×10^{-6} M nisoldipine and/or Ca^{2+}-free solution, in which Na^+ current, Ca^{2+}, the inward rectifier K^+ current (I_{K1}), the electrogenic Na^+-Ca^{2+} exchange current and Na^+-K^+ pump current were eliminated (Tohse *et al.*, 1987). The concentration of intercellular Ca^{2+} was buffered at pCa 8 with 10 mM EGTA. Under these conditions, I_K was measured using a voltage clamp mode. The amplitude of I_K was not affected by 3×10^{-6} M nisoldipine.

The calcium current (I_{Ca}) was recorded in Na^+- and K^+-free solution. The composition of pipette solution was the same when I_K was measured.

The whole-cell clamp method was essentially the same as that described by Hamill *et al.* (1981). The resistance of the pipette filled with internal solution was 1.9–3MΩ. A tight seal was established with Tyrode solution. The perfusate was then changed to the test solution. The voltage drop across the pipette resistance was electrically compensated and voltage pulses were applied to the cell with an interval of 15 s. Current signals were filtered at 500 Hz, digitized at 1 kHz and stored in a computer (NEC 9801 RL) using an on-line data acquisition system.

Results

Effects of histamine on I_K

To examine the effect of histamine receptor stimulation on I_K, various concentrations of histamine were applied to the myocytes. Figure 1a shows current traces in response to various test potentials before and during the superfusion of 10^{-6} M histamine, with the concentration of Ca^{2+} in the pipette equivalent to pCa 8. The I_K was elicited by depolarizing pulses of 500 ms duration to the potentials between − 30 to 90 mV from the holding potential of − 50 mV.

Following the capacitive surge, I_K was activated with a clear delay at its onset and the tail current had two exponential components with time constants of approximately 100 and 300 ms at − 50 mV. Histamine increased I_K at all the test potentials examined. Consequently, the peak amplitude of I_K tail current was enhanced by histamine 3.02 fold when measured upon repolarization from 90 to − 50 mV. The fast and slow components of the I_K were increased almost equally. The two time constants of the tail current, however, were not changed significantly, suggesting that the kinetics of I_K were hardly affected. To examined a possible voltage-dependent action of histamine on I_K, the peak amplitude of the I_K tail current was plotted against the test potential in Figure 1b. Under control conditions, the maximum I_K tail current was at 90 mV, and the potential for a half-maximum amplitude ($V_{0.5}$) was 42 mV. In the presence of histamine, $V_{0.5}$ was shifted in a negative direction by about 10 mV.

The peak amplitude of the I_K tail current was measured upon repolarization from 90 to − 50 mV at various concentrations of histamine and was normalized by the control value. Although the effects of histamine were reversible and reproducible at low concentrations ($< 10^{-7}$ M), they diminished on repetitive application of higher concentrations of the drugs. Higher doses of histamine were therefore used only once in the same cell. The dose-response relationship thus obtained is shown in Figure 2.

The threshold dose of histamine for increasing I_K tail current was near 10^{-9} M and the maximum effect was obtained at about 10^{-6} M. The normalized response of I_K tail current to 10^{-6} M histamine was 3.02 ± 0.24 (mean ± s.e.mean, $n = 4$), and the half-maximum dose was 3.8×10^{-8} M. This value is an order-of-magnitude smaller than

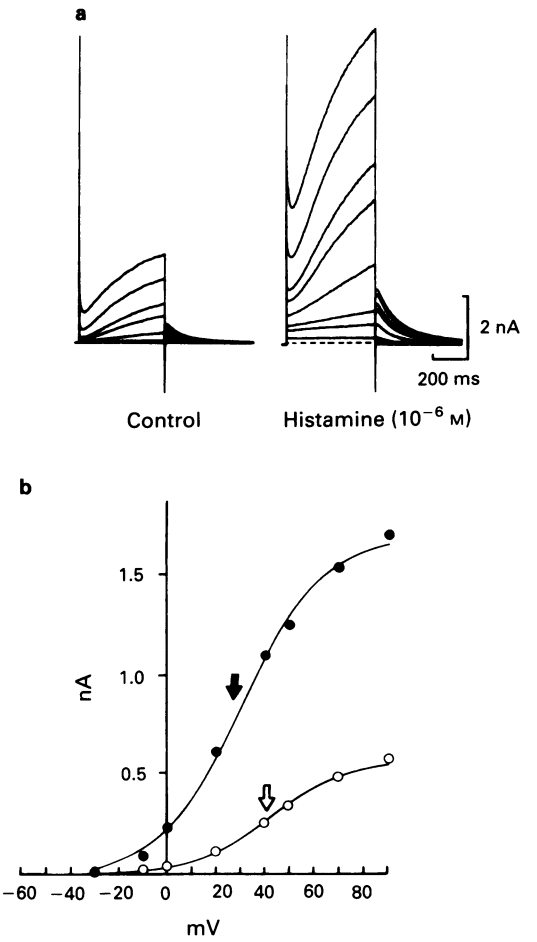

Figure 1 Effect of histamine on I_K. (a) Membrane currents were recorded at various test potentials of − 30, − 10, 0, 20, 40, 50, 70 and 90 mV from the holding potential of − 50 mV, with a duration of 500 ms. The currents on the left are the controls and on the right in the presence of 10^{-6} M histamine. Zero-current level is indicated by dashed lines. (b) I–V relations of I_K tail currents obtained from (a), for control (○) and histamine (●). The increased rate of I_K induced by histamine was greater at lower than at higher test potentials. Both open and closed arrows indicate the potentials for a half maximum amplitude ($V_{0.5}$).

that for histamine on I_K (Tanaka *et al.*, 1991) or on I_{Ca} (Hescheler *et al.*, 1987).

Effects of histamine-receptor blocking agents on I_K

To examine whether the effect of histamine on I_K is mediated by H_1-receptors or H_2-receptors, we investigated the effects of the H_1-antagonist chlorpheniramine and the H_2-antagonist cimetidine on I_K. First, we examined the effect of chlorpheniramine and cimetidine on I_K in the absence of histamine-stimulation (basal I_K). Application of either chlorpheniramine (10^{-7} and 10^{-6} M) or cimetidine (10^{-7}, 10^{-6} and 10^{-5} M) did not affect the basal I_K in four cells, respectively (not shown). We then applied 10^{-7} M or 10^{-6} M chlorpheniramine in the presence of histamine (Kondo *et al.*, 1985; Ahn & Barnett, 1986). Chlorpheniramine at these concentrations did not decrease the I_K tail current, suggesting that histamine stimulation of I_K was not through H_1-receptors.

To find out whether an antagonism occurs between cimetidine and histamine, we applied 10^{-7} M, 10^{-6} M or 10^{-5} M cimetidine in the presence of histamine (Figure 2). On application of 10^{-7} M cimetidine, the dose-response curve for histamine and the I_K value did not change. We then applied histamine in the presence of 10^{-6} M or 10^{-5} M cimetidine

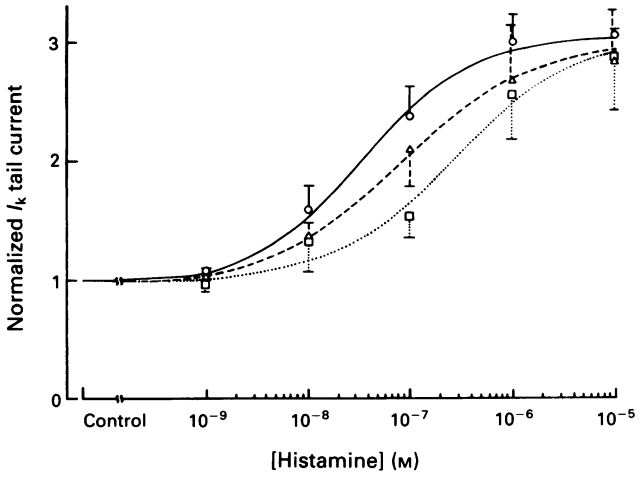

Figure 2 The relationship between histamine added to cimetidine and normalized amplitude of the I_K tail current: mean values (± s.e.mean) of histamine (○), 10^{-6} M cimetidine added to histamine (△) and 10^{-5} M cimetidine in the presence of histamine (□). Number of measurements at each concentration was 4–6. Cimetidine (10^{-7} M) does not displace the dose-response curve which is elicited by histamine only. In these agents, the continuous curves give half-maximum concentrations of 3.8×10^{-8} M, 7.2×10^{-8} M and 2.1×10^{-7} M, respectively.

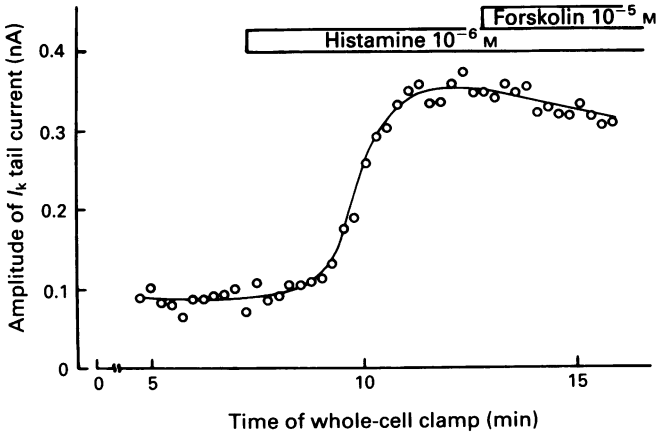

Figure 3 Time course of I_K change produced by forskolin in the presence of histamine. The amplitude of I_K tail current was measured upon repolarization from 20 to − 50 mV. At 1 min after application of 10^{-6} M histamine, I_K began to increase and reached a maximum after 3–4 min. I_K was not increased by the addition of 10^{-5} M forskolin to the histamine.

(Hescheler *et al.*, 1987). In the first case, the threshold dose of histamine for I_K tail current was near 10^{-9} M and the maximum effect was obtained at about 10^{-5} M. The normalized response of the I_K tail current to 10^{-5} M was 2.86 ± 0.63 (mean ± s.e.mean, $n = 4$), and the half-maximum dose was 7.2×10^{-8} M. In the second case, the threshold dose of histamine for I_K tail current was near 10^{-9} M and the maximum effect was obtained at about 10^{-5} M. The normalized response of the I_K tail current to 10^{-5} M was 2.89 ± 0.39 (mean ± s.e.mean, $n = 4$), and the half-maximum dose was 2.1×10^{-7} M. In these conditions, maximum responses of histamine-stimulated I_K tail currents were the same as the value in the absence of cimetidine. The two time constants of the I_K tail current were also uneffected by chlorpheniramine or cimetidine. Cimetidine only appeared to shift the dose-response curve to the right. These results suggest that histamine stimulation of I_K is mainly through H_2-receptors.

Furthermore, this shift shows that the effect of histamine vs cimetidine on I_K is a competitive antagonism. We obtained the pA_2 value from the data shown in Figure 2 by a Schild plot (Schild, 1947). The pA_2 value of cimetidine against histamine was 6.38. This value was similar in potency to cimetidine as an antagonist on the effects of histamine in the human temporal artery (Ottosson *et al.*, 1989) or in the renal artery (Tayo & Bevan, 1986).

Effects of histamine on I_K in the presence of either forskolin or PKI

To examine whether the effect of histamine on I_K is mediated by activation of adenylate cyclase, we investigated the relationship of histamine and forskolin, which is known to activate adenylate cyclase directly. In the experiment shown in Figure 3, we first applied 10^{-6} M histamine, which maximally increased the I_K tail current amplitude, obtained upon repolarization from + 20 to − 50 mV, from 0.086 to 0.355 nA.

After I_K reached the maximum level, 10^{-5} M forskolin, which increased the I_K tail current maximally, was added to the bath. Although I_K tended to decline because of run-down, no clear change in I_K by forskolin was observed. This result was confirmed in four other cells. Thus, the effects of histamine and forskolin are non-additive, suggesting that the effect of histamine is mediated by the activation of adenylate cyclase.

Some studies show that the increase of I_K is mediated by phosphorylation with not only PKA but also protein kinase C (PKC) (Tohse *et al.*, 1987; 1990; Yazawa & Kameyama, 1990; Walsh & Kass, 1988; 1991). To examine whether the effect of histamine on I_K is mainly mediated by PKA, we investigated the effect of histamine in the presence of $PKI_{(5-24)}$, which was known to inhibit PKA directly. In Figure 4, the whole-cell clamp was started with a pipette solution containing 10^{-5} M PKI, and after 11 min, 10^{-8} M histamine was added to the bath.

The time-dependent outward current was increased by a pulse of 500 ms duration to 20 mV from the holding potential of − 50 mV. On the other hand, although I_K tended to decline because of run-down, the amplitude of I_K and its tail was not significantly affected. This result was confirmed in four other cells. In the presence of 10^{-5} M PKI, the increase

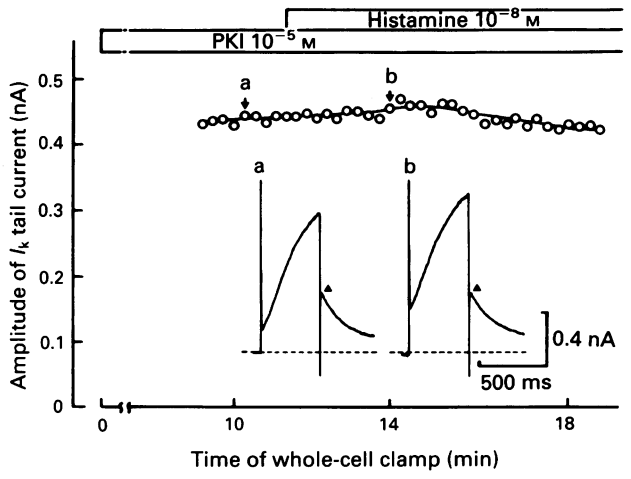

Figure 4 Effect of histamine on I_K in the presence of protein kinase inhibitor (PKI). Time course of the change in the I_K tail current produced by an internal application of 10^{-5} M PKI and 10^{-8} M histamine is illustrated. Test potential was 20 mV and holding potential was − 50 mV. At time zero, whole-cell clamp was established and dialysis of the cell with PKI was started. Inset shows examples of the current traces at the times indicated on the graph (a,b). Dashed lines indicate zero-current level.

measured at the peak of I_K tail current after the application of histamine upon repolarization from 20 to − 50 mV was 5 ± 3% ($n = 5$). By contrast, in the absence of PKI, 10^{-8} M histamine increased the amplitude of the I_K tail by 57 ± 21% ($n = 5$) compared to the control current level. These results suggest that histamine stimulation on I_K is mediated mainly by cyclic AMP-dependent phosphorylation.

Threshold of histamine on Ca^{2+} and K^+ currents

Recently, it has been reported that the threshold of histamine on I_K is different from that on I_{Ca} (Tanaka *et al.*,1991). They have shown that the threshold dose of histamine for increasing the I_K tail current is more than 10^{-8} M. But at this concentration, the previous study reported that I_{Ca} was significantly increased. However, our data showed that the threshold dose of histamine for increasing the I_K tail current was near 10^{-9} M. This dose is less than the value in the above study or the threshold concentration of histamine on I_{Ca} (Hescheler *et al.*, 1987). To examine the threshold dose of histamine on I_{Ca} and I_K, low concentrations ($< 10^{-7}$ M) of histamine were applied externally to the myocytes. In the experiment shown in Figure 5, we superfused the cell with histamine at 10^{-9} M, which is approximately the threshold concentration for affecting the I_K.

This dose increased both the I_K tail from 0.10 to 0.15 nA and the amplitude of I_{Ca} from 0.65 to 0.75 nA. The 10^{-8} M histamine was added to the bath and it increased the I_K tail current to 0.23 nA and I_{Ca} to 0.83 nA. At the concentrations, the time constants or increasing ratio of I_K tail were not significantly different in the external solution which contained 3×10^{-6} M nisoldipine in the absence of Ca^{2+}, 1.8 mM Ca^{2+} without nisoldipine, or 1.8 mM Ca^{2+} added to 3×10^{-6} M nisoldipine. These results exclude the idea that I_K was increased via elevation of the intracellular Ca^{2+} concentration followed by the activation of I_{Ca}. Histamine at 10^{-9} M increased the amplitude of both I_{Ca} and I_K by a factor of 1.37 ± 0.23 (mean ± s.e.mean, $n = 4$) and 1.28 ± 0.13 ($n = 4$) respectively, when they were measured upon depolarization from − 50 to 20 mV.

These results strongly suggest that the threshold doses of histamine for increasing the I_K tail current and the I_{Ca} are similar and the values are near 10^{-9} M.

Discussion

The present study shows that histamine increases I_K by a factor of 3.02. This agent does not affect the kinetics of I_K significantly. Histamine stimulation of I_K is mainly by way of H_2-receptors. Furthermore, the effect of forskolin on I_K is masked by the previous application of histamine, and I_K is little affected by histamine in the presence of internal PKI. These results indicate that the histamine-induced increase of I_K is mediated mainly by cyclic AMP and subsequent activation of PKA.

The threshold dose of histamine for increasing I_K tail current was near 10^{-9} M and the half-maximum dose was 3.8×10^{-8} M. This value is an order-of-magnitude smaller than that previously reported for histamine (Tanaka *et al.*, 1991). The difference in the value found in the previous work and our study may be concerned with both the holding potential and the compositions of the external solutions. Several possibilities are discussed below.

Firstly, several other currents were not eliminated in the previous study as Na^+ and K^+ were present in the test solution. Extracellular K^+ concentration is necessary to activate both inward rectifier K^+ (I_{K1}) and the Na^+-K^+ pump current. Furthermore, activating the Na^+-Ca^{2+} exchange current is dependent on extracellular Na^+. In our study, these other currents were ruled out during the measurement of the amplitude of the I_K tail.

Secondly, the holding potential is concerned with the activation of several currents. It is known that I_K is activated at a more positive than − 40 mV and does not have an inactivation phase. This shows that I_K is already activated when the holding potential is held at more positive than − 40 mV. It is suggested that I_K is already activated at − 40 mV and that in this condition, the holding current is contaminated by the current already activated. I_K is

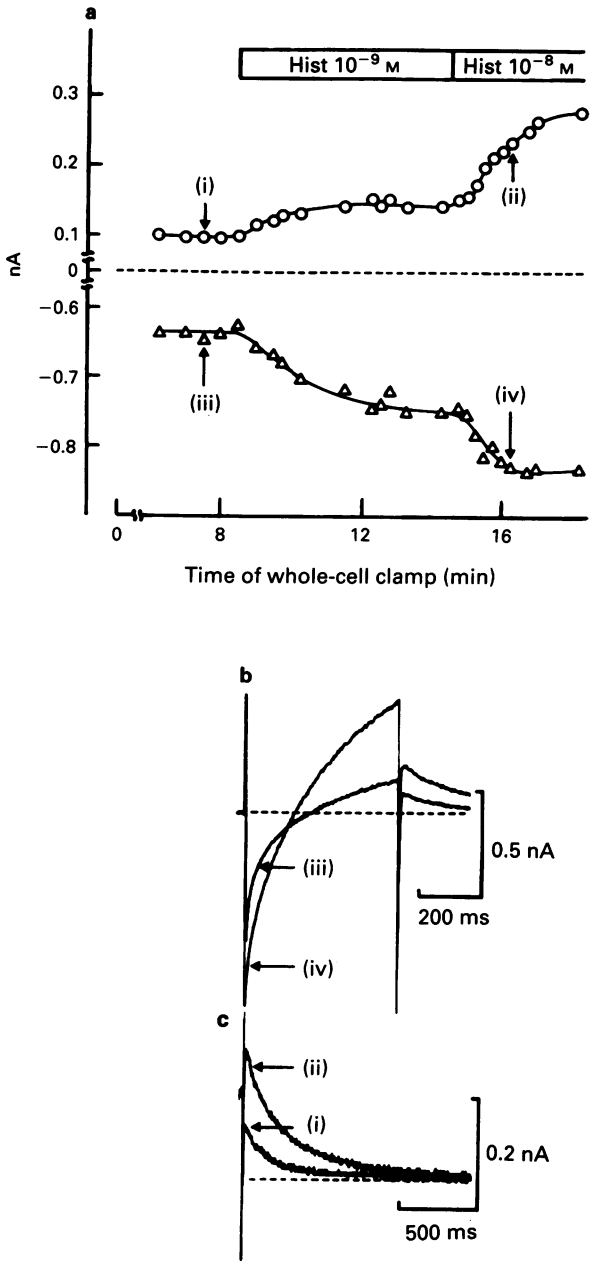

Figure 5 Effect of histamine on I_K and I_{Ca}. (a) Time course of the change in the I_K tail current and I_{Ca} during application of 10^{-9} M and 10^{-8} M histamine is illustrated from 6 min after the start of whole-cell clamp. Holding potential was − 50 mV and the test potential was 20 mV. Both I_K and I_{Ca} were increased in the presence of 10^{-9} M histamine. Dashed line indicates zero-current level. (b) The current traces (iii) in control, (iv) in the presence of 10^{-8} M histamine. When the amplitude of I_{Ca} was measured between peak inward current and the holding current, histamine increased I_{Ca} from 0.65 to 0.83 nA. Zero-current level is indicated by dashed lines. (c) The current traces (i) in control, (ii) in the presence of 10^{-8} M histamine. The amplitude of I_K tail current was measured upon repolarization from 20 to − 50 mV. Histamine increased I_K tail current from 0.10 to 0.23 nA. Zero-current level is indicated by dashed lines.

measured by the difference between the peak of I_K tail current and the holding current. Thus, with the holding potential of -40 mV, a small change in I_K, produced by histamine at near the threshold dose, might be underestimated. The Cl^- current has been recently investigated in guinea-pig ventricular cells (Bahinski *et al.*, 1989; Harvey & Hume, 1989; 1990; Matuoka *et al.*, 1990). The reversal potential for this current depends on the external and internal Cl^- concentrations. In our study, the reversal potential of Cl^- was about -50 mV as predicted by the Nernst equilibrium potential. Unless, the holding potential was around -50 mV, the I_K tail might be contaminated with the Cl^- current.

It has been shown that histamine increases the time-independent Cl^- current in cardiac myocytes (Harvey & Hume, 1990). In the present study, we also observed that a time-independent outward current was activated by a depolarizing pulse following capacitive surge. This current was increased by histamine at or more than 10^{-9} M. This result is consistent with the idea that a Cl^- current is stimulated by histamine with a threshold dose of near 10^{-9} M. However, the time-independent outward current was elicited in the presence of PKI, though I_K was not increased by histamine stimulation (inset of Figure 4). This suggests that the time-independent current might have a component that is not regulated by a cyclic AMP-PKA pathway. Possible mechanisms for this pathway might be a direct coupling of GTP-binding protein to the channel and activation of a protein kinase other than PKA (Yatani & Brown, 1989). It is also possible that the time-independent current is very sensitive to PKA which remains active even in the presence of PKI. Further studies are needed to solve this problem.

Walsh *et al.* (1988) have reported that both isoprenaline and forskolin enhance both I_{Ca} and I_K in guinea-pig ventricular myocytes at 28–32°C, while they increase only I_K at room temperature. In the frog atrial cell, I_K was not increased at room temperature by a concentration of isoprenaline that enhances I_{Ca} (Giles *et al.*, 1989). On the other hand, at a higher temperature (32–37°C), the threshold dose of β-adrenoceptor agents for increasing both I_{Ca} and I_K (I_X) is of a similar value in ventricular cells (Kameyama *et al.*, 1985; Yazawa & Kameyama, 1990), in calf cardiac Purkinje fibres (Kass & Wiegers, 1982). It has been suggested that an underlying mechanism for these effects is phosphorylation of the channel by PKA, although temperature modulates these currents. At a physiological temperature, the threshold dose of isoprenaline for increasing I_K is near 10^{-9} M (Yazawa & Kameyama, 1990), and this value is similar to that for I_{Ca} (Kameyama *et al.*, 1985). Furthermore, the maximum concentrations for increasing the two currents are also in the same range. These results strongly suggest that activation of the common phosphorylation pathway modulates both I_K and I_{Ca} with similar potency at physiological temperatures.

It is well known that histamine, like adrenoceptor stimulants, can produce arrhythmogenic effects on the heart (for review see Wolff & Levi, 1986). Histamine and adrenaline enhance not only I_{Ca} but also I_K, both of which contribute to the action potential duration (APD). When I_K is activated by histamine in working ventricular cells, APD becomes shorter, which may contribute to the ventricular premature conduction or tachycardia.

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