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Histamine H_1-receptor-mediated increase in the Ca^{2+} transient without a change in the Ca^{2+} current in electrically stimulated guinea-pig atrial myocytes

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1 The effects of histamine on the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), action potential and membrane currents were assessed in single atrial myocytes prepared from guinea-pigs.

2 Histamine caused a concentration-dependent increase in the $[Ca^{2+}]_i$ transient in indo1/AM loaded myocytes when stimulated electrically at 0.5 Hz. However, the maximum increase in $[Ca^{2+}]_i$ transient produced by histamine was less than 50% of that elicited by isoprenaline. The histamine-induced increase in $[Ca^{2+}]_i$ transient was significantly inhibited by chlorpheniramine, but not by cimetidine.

3 Pretreatment with nifedipine nearly completely suppressed the histamine-induced increase in $[Ca^{2+}]_i$ transient. Cyclopiazonic acid did not affect the histamine response.

4 In the whole-cell current-clamp mode of the patch-clamp method, both histamine and isoprenaline prolonged action potential duration (APD) in atrial myocytes. In the presence of Co^{2+} or nifedipine, the isoprenaline-induced APD prolongation was abolished and an APD shortening effect was manifested, while histamine still increased APD. The APD prolongation elicited by histamine was reversed by chlorpheniramine.

5 In the voltage-clamp mode, the histamine-sensitive membrane current was inwardly rectifying and reversed close to the calculated value of the K^+ equilibrium potential. Histamine had no apparent effect on L-type Ca^{2+} current, in contrast to the pronounced effect of isoprenaline.

6 These results indicate that in guinea-pig atrial myocytes stimulation of H_1-receptors with histamine does not directly activate Ca^{2+} channels but causes an elevation of $[Ca^{2+}]_i$ transient by increasing Ca^{2+} influx through the channels during the prolonged repolarization of action potentials resulting from inhibition of the outward K^+ current.

Keywords: Histamine; H_1-receptors; intracellular Ca^{2+} concentration; action potential; L-type Ca^{2+} current; guinea-pig atrial cells

Introduction

Histamine produces a positive inotropic effect in most mammalian cardiac muscles. There is a species difference in the subtype of histamine receptors involved in the genesis of the positive inotropic effect, and, in addition, different subtypes mediate the effect even in different parts of the heart within the same species. The positive inotropic effect of histamine in guinea-pig ventricular myocardium is predominantly mediated by H_2-receptors (Verma & McNeill, 1977; Hattori *et al*., 1994), whereas that in rabbit ventricular myocardium is predominantly by H_1-receptors (Hattori *et al*., 1988b, 1990, 1994). In contrast, the receptors through which the effect is produced in atrial muscles are entirely of the H_1-subtype in the guinea-pig (Reinhardt *et al*., 1974; Steinberg & Holland, 1975; Verma & McNeill, 1977) and of the H_2-subtype in the rabbit (Hattori *et al*., 1988b, 1991). On the other hand, in the monkey only H_2-receptors mediate the positive inotropic effect of histamine in both atrium and ventricle (Hattori *et al*., 1983).

The positive inotropic effect elicited by stimulation of H_2-receptors is most likely to be principally due to an increase in intracellular cyclic AMP (Verma & McNeill, 1977; Reinhardt *et al*., 1977), a mechanism analogous to that well known for β-adrenoceptors. Thus, increased intracellular cyclic AMP activates protein kinase A; this could promote Ca^{2+} influx through phosphorylation of sarcolemmal L-type Ca^{2+} channels (Trautwein & Hescheler, 1990) and lead to a large amount of Ca^{2+} release from the sarcoplasmic reticulum in which Ca^{2+} uptake is increased through phosphorylation of phospholamban (Tada & Katz, 1982), thereby enhancing myocardial contraction. On the contrary, the mechanism by which stimulation of H_1-receptors produces a positive inotropic effect is poorly understood. With respect to the intracellular messenger(s) generated by activation of H_1-receptors, the acceleration of the hydrolysis of phosphoinositides, with resultant production of inositol 1,4,5-trisphosphate and diacylglycerol, has been postulated to be responsible for the positive inotropic effect (Sakuma *et al*., 1988). However, we have found that the phospholipase *C* inhibitors 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate and neomycin did not modify the positive inotropic effect of histamine while inhibiting effectively the histamine-induced phosphoinositide hydrolysis in guinea-pig left atrium (Hattori *et al*., 1989), implying a dissociation of the positive inotropic effect from the phosphoinositide hydrolysis in response to H_1-receptor stimulation. Thus, the role of the phosphoinositide hydrolysis products as principal mediators for the H_1-receptor-mediated positive inotropic effect is currently unsettled. Electrophysiological studies have demonstrated that stimulation of H_1-receptors increases action potential duration (APD) in guinea-pig left atrial muscle (Amerini *et al*., 1982; Borchard & Hafner, 1986; Hattori *et al*., 1988a). The H_1-receptor-mediated increase in APD seems unlikely to be attributable to enhancement of the L-type Ca^{2+} current (I_{Ca}). This notion has been indirectly

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evidenced by previous observations that stimulation of H_1-receptors with histamine fails to restore the electrical responses in K^+-depolarized guinea-pig left atrium (Inoue *et al*., 1979; Mantelli *et al*., 1982). Nevertheless, the positive inotropic effect of histamine in guinea pig left atrium is greatly suppressed in the presence of the Ca^{2+} channel antagonists nifedipine and nisoldipine (Hattori *et al*., 1988a). Therefore, it may be inferred that the increase in Ca^{2+} influx through Ca^{2+} channels as a result of APD prolongation would contribute to the positive inotropic effect of H_1-receptor stimulation.

To gain further insight into the possible mechanism underlying the positive inotropic effect of H_1-receptor activation, we investigated the effect of histamine on the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) transient and I_{Ca} in single atrial myocytes isolated from guinea-pigs. No study has been done to assess changes in the $[Ca^{2+}]_i$ transient and I_{Ca} in response to H_1-receptor stimulation in cardiomyocytes. This paper is the first report showing direct evidence that activation of H_1-receptors with histamine causes an elevation of $[Ca^{2+}]_i$ in electrically stimulated cardiomyocytes without increasing directly I_{Ca}.

Methods

Isolation of atrial myocytes

Guinea-pigs of either sex, weighing 250–300 g, were anaesthetized with sodium pentobarbital (50–60 mg kg^{-1} i.p.). The heart was quickly dissected from the open-chest animal that was ventilated with an artificial respirator. The animal was cannulated and perfused by a Langendorff apparatus. The heart was retrogradely perfused with a modified Tyrode solution at a temperature of 36°C until its beating rate became stable. The composition of the solution (pH 7.4) was (in mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 0.5, NaH_2PO_4 0.33, HEPES 5.0 and glucose 5.5. Then, the perfusate was changed to a nominally Ca^{2+}-free Tyrode solution for 5 min, resulting in cessation of the heart beat. The quiescent heart was perfused with a nominally Ca^{2+}-free Tyrode solution containing collagenase (0.03% wt/vol; Wako Pure Chemical, Osaka, Japan) for 12 min. The collagenase solution was washed out with KB solution which contained 70 mM KOH, 50 mM L-glutamic acid, 40 mM KCl, 20 mM taurine, 20 mM KH_2PO_4, 3.0 mM $MgCl_2$, 10 mM glucose, 0.5 mM EGTA, 10 mM HEPES (pH 7.4), and 1% bovine serum albumin. The atrial tissue was cut into small pieces, agitated gently in a small beaker filled with KB solution, and then filtered through a 100 μm stainless steel mesh.

Measurement of indo-1 fluorescence

Single atrial myocytes bathed in KB solution were loaded with the fluorescent Ca^{2+} probe indo-1 by incubation with the acetoxymethyl ester of indo-1 (5 μM indo-1/AM; Dojin, Kumamoto, Japan) and 0.02% Pluronic F-127 (Molecular Probes, Eugene, OR, U.S.A.) for 7.5 min at room temperature, followed by washing with KB solution for 60 min. Small aliquots of loaded myocytes were placed in the experimental chamber filled with Tyrode solution, allowed to settle for 5 min, and superfused with Tyrode solution for at least 15 min. Then, myocytes were field stimulated at a rate of 0.5 Hz by a pair of platinum electrodes connected to an electronic stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan) through an isolation unit (SS-104J, Nihon Kohden).

The microfluometry system (OSP100-CA, Olympus, Tokyo, Japan) was used to provide and control the ultraviolet light of 360 nm with a monochromator for excitation of indo-1 from a 75 W xenon arc lamp. The excitation light beam was directed into an invert microscope (IX-70, Olympus) equipped for epifluorescence measurements. Emitted fluorescence signals from single indo-1/AM loaded mycotyes were digitalized at 200 Hz, and the ratio of fluorescence emission of 410 nm to that at 485 nm was recorded. The ratio of indo-1 emission at the two wavelengths was calculated after subtracting the background autofluorescence. Our results with indo-1/AM-loaded myocytes are expressed as the fluorescence ratio rather than as absolute Ca^{2+} concentration, because intracellular binding and compartmentalization of this indicator prevent from obtaining accurate *in vivo* calibrations (Spurgeon *et al*., 1990).

The experiments were implemented at a temperature of 23°C to minimize loss of the Ca^{2+} indicator from myocytes. The fluorescence ratio data were processed and stored in an IBM/AT-type microcomputer using a software (OSP-SFCA, Olympus).

Measurements of action potential and membrane currents

Electrophysiological recordings were performed by the whole-cell patch-clamp technique, using glass patch electrodes with a resistance of 2–5 MΩ. Membrane currents were recorded in voltage-clamp mode, and action potentials were recorded using the current-clamp version of patch clamping with a patch-clamp amplifier (CEZ 2300, Nihon Kohden). The signals were displayed on an oscilloscope (2221A, Sony-Tektronix, Tokyo, Japan) and were simultaneously fed to the data recorder system, consisting of a video cassette recorder (NV-F1, National, Osaka, Japan) and a PCM converter system (PCM-501ES, Sony, Osaka, Japan) and a PCM converter system (PCM-501ES, Sony, Osaka, Japan) as a back-up. The current and voltage signals were filtered at 1 kHz digitalized by a AD converter (ADX-98, Canoopus Electronics, Kobe, Japan) at 2 kHz, and stored in the 20 MByte hard disk of a personal computer (PC-98RL, NEC, Tokyo, Japan) for later analysis.

Whole-cell membrane currents were measured using a voltage ramp protocol applied from a holding potential of −40 V. The voltage ramp protocols ($dV/dt = \pm 0.9$ V s^{-1}) consisted of three phases: an initial +80 mV depolarizing phase from a holding potential of −40 mV, a second hyperpolarizing phase of 160 mV, and then a third phase returning to the holding potential. The current-voltage relationship was measured during the second hyperpolarizing potential. The current-voltage relationship was measured during the second hyperpolarizing phase. The averages of two to three consecutive current-voltage relationships are demonstrated in the figure. The pipette contained (in mM): K-aspartate 100, KCl 20, $MgCl_2$ 1.0, K_2-ATP 5.0, K_2-creatine phosphate 5.0, EGTA 10 and HEPES 5.0; pH 7.4 with KOH. The final K^+ concentration in the pipette solution was 150 mM. The external bathing solution was a modified Tyrode solution.

I_{Ca} was elicited by 300-ms depolarizing test pulse to +10 mV from a holding potnetial of −40 mV in order to avoid the Na^+ and T-type Ca^{++} currents. The compositions of the external and internal solutions were formulated to eliminate the involvement of K^+ currents in the whole-cell membrane currents. Thus, the composition of the internal solution (pH 7.4) was (in mM): Cs-aspartate 110, CsCl 20,

$MgCl_2$ 5.0, Na_2-creatine phosphate 5.0, EGTA, 10 and HEPES 10, while that of the external solution (pH 7.4) was (in mM): NaCl 143, CsCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.5, NaH_2PO_4 0.33, glucose 5.5 and HEPES 5.5. Myocytes that exhibited the clear run-down within the first 10 min were discarded. To minimize the influence of the rundown, the time window between 10 and 30 min after the initial recording was chosen to measure I_{Ca} with respect to drug effects. The amplitude of I_{Ca} was measured as the difference between peak of the inward current and current at the end of the test pulse. The temperature of perfusate was kept constant at 36 ± 1°C.

Drugs

The compounds used are as follows: histamine dihydrochloride (Merck, Darmstadt, Germany); (−)-isoprenaline hydrochloride, nifedipine and cyclopiazonic acid (Sigma Chemical, St. Louis, Missouri, U.S.A.); (+)-chlorpheniramine maleate (Schering, Osaka, Japan); and cimetidine (Fujisawa, Osaka, Japan). All drugs except nifedipine and cyclopiazonic acid were dissolved in distilled water. Nifedipine was prepared as a stock solution (10 mM) in absolute ethanol. Cyclopiazonic acid was dissolved in dimethyl sulphoxide. Further dilutions were made with Tyrode solution. The experiments with nifedipine were performed in the dark and the solution bottles and tubing were covered with aluminium foil for further security against degradation.

Statistical analysis

All values are presented in terms of means ± s.e.mean. Analysis by Student's *t*-test was performed for paired or unpaired observations. *P* values of less than 0.05 was considered significant.

Results

Effects on $[Ca^{2+}]_i$ transient

Figure 1A shows representative tracings of changes in the $[Ca^{2+}]_i$ transient after application of 1 μM histamine and 100 nM isoprenaline recorded from an electrically stimulated single guinea-pig atrial myocyte. In myocytes stimulated electrically at 0.5 Hz, both of the agonists caused an increase in $[Ca^{2+}]_i$ transient. The increase in $[Ca^{2+}]_i$ transient induced by histamine attained a steady level at 3 min after its addition, whereas that by isoprenaline took a longer time ($\geqslant$5 min) to attain its maximum (Figure 1B). Furthermore, the $[Ca^{2+}]_i$ response to histamine was less pronounced than that to isoprenaline in the same myocyte. When the response to 100 nM isoprenaline in a given myocyte was taken as 100%, the increase in $[Ca^{2+}]_i$ transient induced by 1 μM histamine was only $41 \pm 6\%$ ($n=4$).

Figure 2 shows the effects of various concentrations of histamine on $[Ca^{2+}]_i$ transient. A significant increase in $[Ca^{2+}]_i$ transient occurred at concentrations higher than 1 nM. A maximum response was observed at $\geqslant$1 μM. Thus, the concentration required to produce 50% of the maximal $[Ca^{2+}]_i$ response (EC_{50}) was approximately 26.3 nM. The effect of 1 μM histamine on the $[Ca^{2+}]_i$ transient was significantly inhibited by the H_1-antagonist chlorpheniramine at 1 μM, but remained unchanged in the presence of the H_2-antagonist cimetidine at 10 μM (Figure 3). These antagonists at concentrations used in this study had a negligible effect on $[Ca^{2+}]_i$ transient in the absence of histamine.

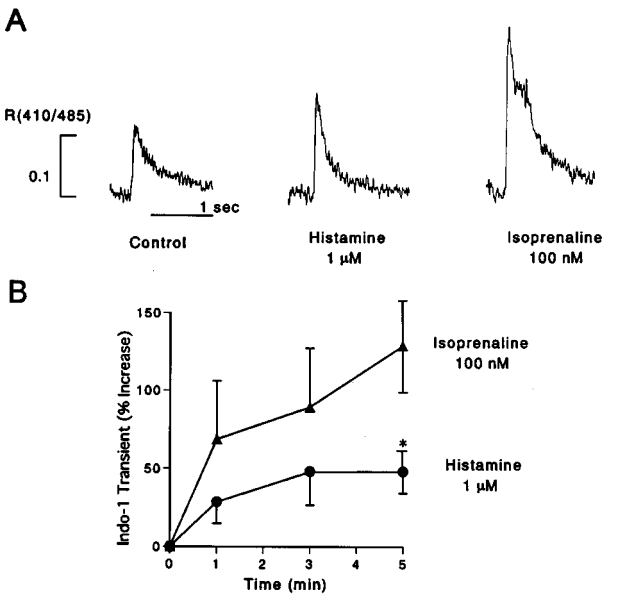

Figure 1 Effects of histamine and isoprenaline on $[Ca^{2+}]_i$ transient in guinea-pig atrial myocytes stimulated electrically at 0.5 Hz. (A) Representative tracings showing $[Ca^{2+}]_i$ transients before and 5 min after application of 1 μM histamine or 100 nM isoprenaline. (B) The time course of changes in $[Ca^{2+}]_i$ transient after application of 1 μM histamine and 100 nM isoprenaline. The points are expressed as a percentage of the values recorded before the drug addition and are shown as means ± s.e.mean of four experiments. $*P<0.05$ *vs* the corresponding value with isoprenaline.

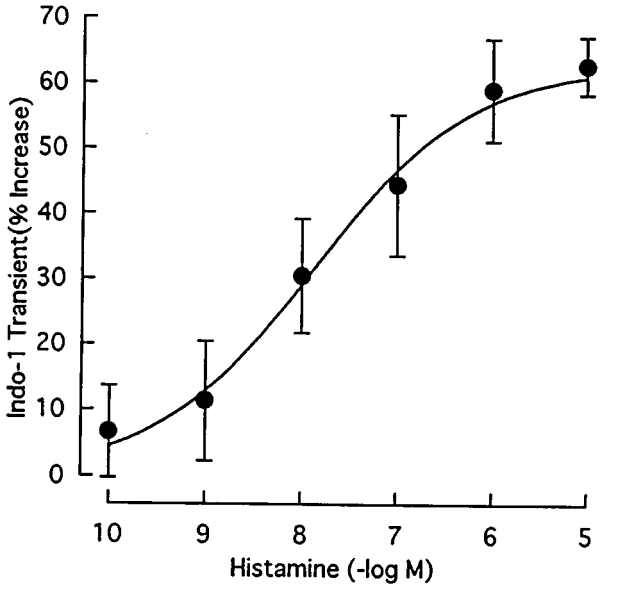

Figure 2 Concentration-response curve for the effect of histamine on $[Ca^{2+}]_i$ transient in guinea-pig atrial myocytes stimulated electrically at 0.5 Hz. The points are expressed as a percentage of the values recorded before application of histamine and are shown as means ± s.e.mean of four to 16 experiments.

The Ca^{2+} channel antagonist nifedipine at 1 μM decreased the $[Ca^{2+}]_i$ transient by $26 \pm 2\%$ ($n=10$). Pretreatment with nifedipine nearly completely eliminated the increasing effect of histamine on $[Ca^{2+}]_i$ transient (Figure 4). Thus, the increase in $[Ca^{2+}]_i$ transient induced by 1 μM histamine was $47 \pm 2\%$ ($n=10$) in the absence and $2 \pm 1\%$ ($n=10$) in the presence of 1 μM nifedipine. On the other hand, pretreatment with 10 μM

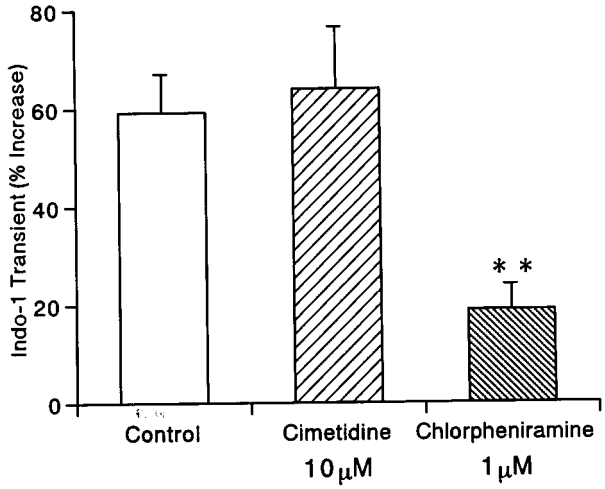

Figure 3 Influences of cimetidine and chlorpheniramine on the increase in $[Ca^{2+}]_i$ transient induced by histamine in guinea-pig atrial myocytes stimulated electrically at 0.5 Hz. The myocytes were treated for 5 min with 10 μM cimetidine or 1 μM chlorpheniramine, which remained in the bath solution during exposure to 1 μM histamine. The bars are expressed as a percentage of the values recorded before the application of 1 μM histamine and are shown as means ± s.e.mean of five to seven experiments. $^{**}P<0.01$ *vs* the value with histamine alone.

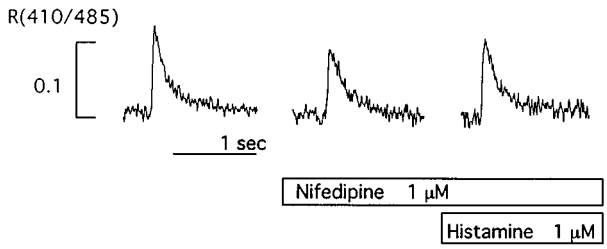

Figure 4 Influence of nifedipine on the increase in $[Ca^{2+}]_i$ transient induced by histamine in guinea-pig atrial myocytes stimulated electrically at 0.5 Hz. The addition of 1 μM nifedipine decreased $[Ca^{2+}]_i$ transients, which attained a steady level within 5 min, and then 1 μM histamine was applied in the presence of nifedipine.

cyclopiazonic acid, a selective inhibitor of the Ca^{2+}-pump ATPase of the sarcoplasmic reticulum (Seidler *et al*., 1989), had no inhibitory effect on the histamine-induced increase in $[Ca^{2+}]_i$ transient (58 ± 17%, $n=6$).

Effects on action potentials

When action potentials in atrial myocytes were elicited by current injection at a rate of 0.1 Hz, 1 μM histamine and 100 nM isoprenaline both increased APD without affecting the resting membrane potential (Figure 5). The effect of isoprenaline in increasing the APD was more marked at the 50% repolarization level (APD_{50}) than at the 90% repolarization level (APD_{90}). Thus, isoprenaline prolonged APD_{50} and APD_{90} by 106 ± 13% and 57 ± 6%, respectively ($n=4$). On the other hand, when histamine was applied, APD_{50} and APD_{90} were prolonged by 66 ± 16% and 59 ± 10%, respectively ($n=4$). The APD prolongation elicited by 1 μM histamine was reversed by 1 μM chlorpheniramine (Figure 6).

In the presence of 2 mM Co^{2+}, isoprenaline shortened rather than prolonged APD (Figure 7B). A similar APD shortening effect of isoprenaline was observed in the presence of 1 μM nifedipine (Figure 7B). In contrast, histamine

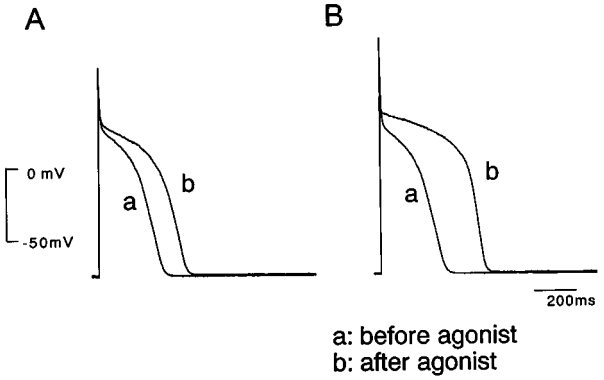

Figure 5 Effects of histamine (A) and isoprenaline (B) on action potentials in guinea-pig atrial myocytes. Action potentials were elicited by current injection at a rate of 0.1 Hz. Superimposed records before and 3 min after application of 1 μM histamine or 100 nM isoprenaline are shown.

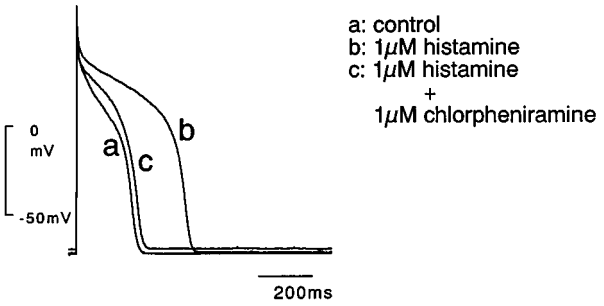

Figure 6 Reversal effect of chlorpheniramine on the APD prolongation induced by histamine in guinea-pig atrial myocytes. Superimposed action potentials before any drug treatment, 3 min after the addition of 1 μM histamine and 5 min after the further addition of 1 μM chlorpheniramine are shown.

increased APD even in the presence of Co^{2+} or nifedipine (Figure 7A).

Histamine-sensitive membrane current

Changes in the whole-cell current were monitored by applying a triangular voltage ramp ($dV/dt = \pm 0.9$ V s^{-1}) between +40 and −120 mV from a holding potential of −40 mV every 10 s. Figure 8A illustrates the current-voltage relationships of the membrane currents recorded before and 3 min after exposure to 10 μM histamine. The histamine-sensitive current, obtained by the digital substraction of the current trace in the presence of histamine from that in its absence, clearly showed inward rectification (Figure 8B). The reversal potential of this current averaged −86.7 ± 2.7 mV ($n=3$), close to the calculated value of the K^+ equilibrium potential ($E_K = -88.9$ mV). Thus, the histamine-sensitive component of the currents evoked by the voltage ramp is thought to be carried principally by K^+-selective ion channels.

Effects on I_{Ca}

Typical tracings of the effects of 1 μM histamine and 100 nM isoprenaline on I_{Ca} elicited by a depolarizing pulse from a holding potential of −40 mV to +10 mV in atrial myocytes are depicted in Figure 9. The addition of isoprenaline caused a 5.5 fold increase in I_{Ca} ($n=4$). In contrast, histamine failed to produce a significant effect on I_{Ca} (5 ± 9%, $n=4$). Also, the

time integral of the current was not significantly changed by histamine (111 ± 9% of control, $n = 4$).

Discussion

In the present study, we demonstrated that histamine increased the $[Ca^{2+}]_i$ transient in indo-1/AM loaded and electrically stimulated atrial myocytes from guinea-pigs. The increase in the $[Ca^{2+}]_i$ transient induced by histamine was blocked by chlorpheniramine, but remained unchanged with cimetidine, which implies mediation through H_1-receptors. Pretreatment with nifedipine eliminated the histamine-induced increase in $[Ca^{2+}]_i$ transient. The finding that the effect of histamine on $[Ca^{2+}]_i$ transient was unaffected by pretreatment with cyclopiazonic acid rules out the possibility that histamine might have acted at the sarcoplasmic reticulum to potentiate Ca^{2+}-induced Ca^{2+} release, which could be indirectly inhibited by the I_{Ca} block with nifedipine. Thus, the observed increase in the amplitude of $[Ca^{2+}]_i$ transient by histamine is likely to be entirely due to the increase in Ca^{2+} influx *via* Ca^{2+} channels. We have previously shown that the postive inotropic effect of activation of H_1-receptors with histamine in guinea-pig left atrium was drastically suppressed in the presence of nifedipine (Hattori *et al*., 1988a). Therefore, it would be reasonable to conclude that an increased amplitude of $[Ca^{2+}]_i$ transient is a main mechanism for the positive inotropic effect of H_1-receptor stimulation.

In current-clamp mode, histamine markedly prolonged APD in guinea-pig atrial cells. The prolongation of APD was mediated by H_1-receptors, since the addition of chlorpheniramine reversed it. The APD prolongation in response to H_1-receptor stimulation is consistent with the results obtained in the multicellular preparations (Amerini *et al*., 1982; Borchard & Hafner, 1986; Hattori *et al*., 1988a). Isoprenaline also

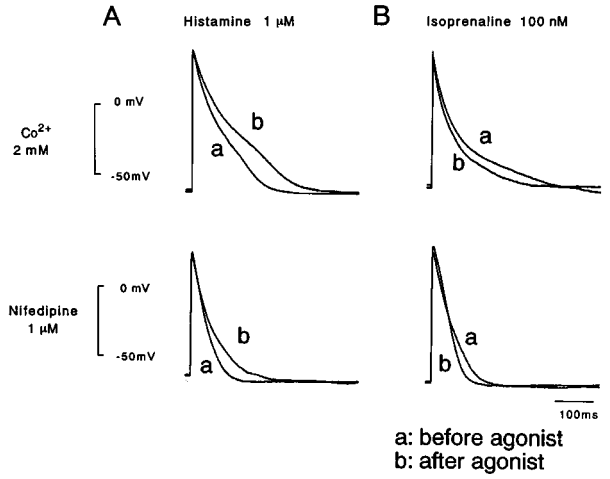

Figure 7 Effects of histamine (A) and isoprenaline (B) on action potentials in the presence of Co^{2+} (*upper panels*) or nifedipine (*lower panels*) in guinea-pig atrial myocytes. The myocytes were treated for 2 min with 2 mM Co^{2+} or 1 μM nifedipine, which remained in the bath solution during exposure to 1 μM histamine or 100 nM isoprenaline. Superimposed records before and after application of each agonist are shown.

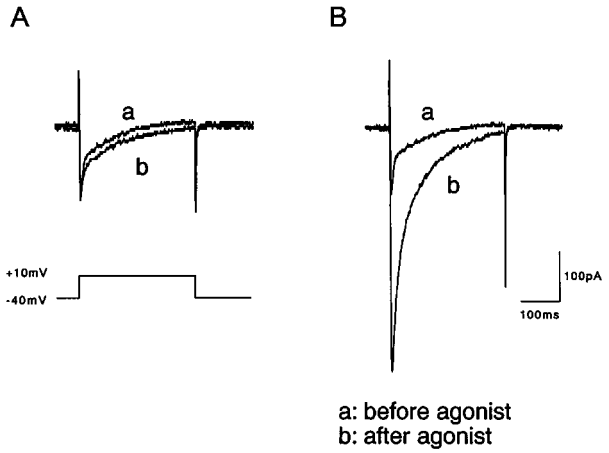

Figure 9 Effects of histamine (A) and isoprenaline (B) on I_{Ca} in guinea-pig atrial myocytes. I_{Ca} was elicited by 300-ms depolarizing test pulse to +10 mV from a holding potential of −40 mV. Current traces before and 3 min after exposure to 1 μM histamine or 100 nM isoprenaline are superimposed.

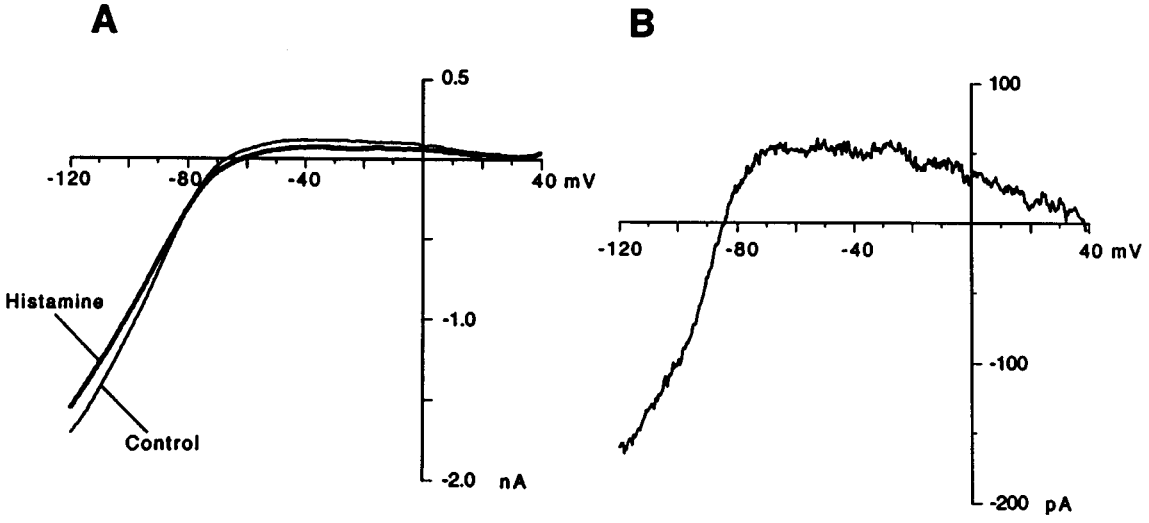

Figure 8 Effect of histamine on the whole-cell current response to voltage ramps applied every 10 s in guinea-pig atrial myocytes. (A) Current-voltage relationships recorded before and 3 min after exposure to 10 μM histamine. (B) Current-voltage relationship of the histamine-sensitive current, obtained by substraction, as indicated in the text.

increased APD in atrial cells. However, whereas histamine had an equal effect on APD_{50} and APD_{90}, the effect of isoprenaline was more marked on APD_{50} than APD_{90}. Furthermore, the isoprenaline-induced APD prolongation was abolished in the presence of Co^{2+} or nifedipine. Thus, it is most likely that the APD prolongation induced by isoprenaline results from an increase in I_{Ca}. The APD shortening effect of isoprenaline observed in the presence of Co^{2+} or nifedipine appears to be attributable to the enhancement of the delayed outward rectifier K^+ current (Kameyama *et al*., 1985; Yazawa & Kameyama, 1990) and/or activation of the Cl^- current (Harvey & Hume, 1989). On the other hand, histamine still produced an increase in APD in the presence of Co^{2+} or nifedipine. When K^+ channel currents were eliminated in Cs^+-rich, K^+-free medium, histamine did not significantly affect I_{Ca}, in contrast to the markedly increasing effect of isoprenaline. Therefore, the prolongation of APD seems to be ascribed to modulation by H_1-receptor stimulation of membrane current(s) other than I_{Ca}.

We have previously found that the Na^+ channel blocker tetrodotoxin has no effect on the APD prolongation by histamine in guinea-pig left atrium (Hattori *et al*., 1988a). This excludes the possibility that stimulation of H_1-receptors modulates the Na^+ current system and thereby prolongs APD. Thus, it could be considered that a decrease in outward K^+ current may contribute to the H_1-receptor-mediated increase in APD. The present voltage clamp experiments revealed that histamine was able to act on an inwardly rectifying current that reversed close to the calculated value of E_K, indicating that histamine inhibits an outward K^+ current in guinea-pig atrial myocytes. In recent work, we have shown that histamine decreases the basal activity of acetylcholine-activated K^+ channels in guinea-pig atrial myocytes which is mediated by H_1-receptors (Tohse *et al*., 1995). In as much as acetylcholine-activated K^+ channels play an important role in the repolarization of action potentials in atrial cells (Kaibara *et al*., 1991; Carmeliet, 1994), a plausible explanation for the APD prolongation caused by H_1-receptor stimulation may be the inhibition of the background K^+ current that is produced by basal activity of acetylcholine-activated K^+ channels. However, it has to be considered that H_1-receptors may couple to another K^+ channel and its inhibition may result in the APD prolongation. Whatever the outward current responsible for the APD prolongation, our present results strongly suggest that in guinea-pig atrial myocytes activation of H_1-receptors with histamine causes an increase in Ca^{2+} influx through Ca^{2+} channels during the prolonged plateau phase of action potentials and results in increased $[Ca^{2+}]_i$ transient, leading to a positive inotropic action.

The most important new finding of this study is that stimulation of H_1-receptors produced a modest increase in the amplitude of $[Ca^{2+}]_i$ transient in electrically stimulated guinea-pig atrial myocytes. However, this cannot be the only mechanism involved in the positive inotropic action of H_1-receptor stimulation. The increase in the amplitude of $[Ca^{2+}]_i$ transient was much smaller than that produced by isoprenaline. Nevertheless, we have found that the maximum positive inotropic effect of histamine is comparable to that of isoprenaline in guinea-pig left atrium (Hattori *et al*., 1989, 1993). Furthermore, the EC_{50} value for the effect of histamine on force of contraction that has been reported previously in guinea-pig left atrium (209 nM; Hattori *et al*., 1989) was about ten times higher than the EC_{50} for the effect on $[Ca^{2+}]_i$ transient obtained in this study. We interpret this to indicate that the positive inotropic effect of histamine at high concentrations in guinea-pig left atrium may involve, in part, an apparent sensitization of the myofilaments to Ca^{2+}. We have previously demonstrated that activation of H_1-receptors with histamine causes a positive inotropic effect without producing APD prolongation in rabbit papillary muscle, because APD could be decreased by concomitant activation of H_2-receptors (Hattori *et al*., 1990). Thus, the positive inotropic effect of H_1-receptor stimulation in rabbit papillary muscle may be in large part due to an increased myofibrillar Ca^{2+} sensitivity. An increase in the Ca^{2+} responsiveness of the myofilaments has been implicated as one of the mechanisms responsible for the positive inotropic effects of α_1-adrenoceptor agonists and endothelin-1 (Endoh & Blinks, 1988; Wang *et al*., 1991). These receptor agonists stimulate Na^+-H^+ exchange and thereby causes an intracellular alkalinization in cardiomyocytes (Iwakura *et al*., 1990; Krämer *et al*., 1991). This increase in intracellular pH may explain the increase in the Ca^{2+} responsiveness of the myofilaments. Whether the same mechanism could be responsible for the increase in myofibrillar Ca^{2+} sensitivity in response to H_1-receptor stimulation or whether other effects on the myofilaments may be involved as well remains to be determined. However, it is of interest to note a striking similarity between characteristic of the positive inotropic effect produced by H_1-receptor stimulation and those by α_1-adrenoceptor agonists as well as endothelin-1; the ability of H_1-receptor stimulation to prolong the duration of a single contraction is very similar to those of α_1-adrenoceptor agonists and endothelin-1 (Mantelli *et al*., 1982; Endoh & Blinks, 1988; Hattori *et al*., 1993).

In conclusion, activation of H_1-receptors with histamine caused an increase in the $[Ca^{2+}]_i$ transient in electrically stimulated single atrial myocytes from guinea-pigs. Although this increase in $[Ca^{2+}]_i$ transient appeared to be related to Ca^{2+} influx *via* a nifedipine-sensitive pathway, histamine failed to enhance directly I_{Ca} in the myocytes. We suggest therefore that an increase in Ca^{2+} influx through Ca^{2+} channels as a result of the prolongation of APD possibly due to inhibition of the outward K^+ current may contribute to the increased $[Ca^{2+}]_i$ transient. However, the subcellular pathways that transduce the signal to the ionic mechanism underlying the APD prolongation by H_1-receptor stimulation remained to be elucidated.

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