The Effects of Histamine on Contraction Frequency, Sodium Influx, and Cyclic AMP in Cultured Rat Heart Cells

David McCall and Charles Y. Lui

Histamine has been shown to have both positive inotropic and chronotropic effects. To evaluate these effects, spontaneously contracting monolayers of cultured rat myocardial cells were treated with histamine, $10^{-7}$ M–$10^{-4}$ M. This resulted in a dose-dependent increase in contraction frequency reaching a maximum in $10^{-5}$ M histamine. Contraction frequency (mean ± SEM) increased from a control of $121 \pm 5$ contractions per minute to $153 \pm 4.5, 212 \pm 4$, and $216 \pm 1$ in $10^{-7}$ M, $10^{-6}$ M, $10^{-5}$ M, and $10^{-4}$ M histamine, respectively (for each n = 10, p < 0.001). The effect was time-dependent, taking 30 minutes to develop fully. Changes in contraction frequency were accompanied by parallel dose- and time-dependent increases in the verapamil-sensitive sodium influx. Verapamil-sensitive sodium influx (pmol/cm$^2$/sec) increased from a control of $10.45 \pm 1.44$ (mean ± SEM) to $24.34 \pm 2.41$ and $32.57 \pm 2.35$ at 10- and 30-minute treatment with $10^{-6}$ M histamine (n = 5, p < 0.001). These data fit the previously described relation between verapamil-sensitive sodium influx and contraction frequency in these cells. Cimetidine ($10^{-4}$ M) but not diphenhydramine ($10^{-4}$ M) abolished both the contraction frequency and sodium influx response to histamine. Subsequent studies showed a dose- and time-dependent elevation of cyclic adenosine monophosphate (cAMP) with histamine treatment. Cell cAMP (control = 5.25 pmol/mg protein) increased by 30%, 57%, 94%, and 224% in $10^{-7}$ M, $10^{-6}$ M, $10^{-5}$ M, and $10^{-4}$ M histamine, respectively, the changes between $10^{-7}$ M and $10^{-5}$ M closely paralleled the changes in beating rate and sodium influx. The histamine effect on cAMP was competitively inhibited by cimetidine. The results suggest histamine increases beating rate by increasing verapamil-sensitive sodium influx and that the effects are mediated by $H_2$ receptors coupled to adenylyl cyclase. (Circulation Research 1986;59:668–675)
dominate while in the human\textsuperscript{15-17} all effects represent \( H_2 \)-mediated responses. In both the cat\textsuperscript{18} and the rat\textsuperscript{19} neither \( H_1 \) nor \( H_2 \) receptors appear to mediate histamine effects. In these species the stimulatory effect of histamine appeared to result indirectly from release of endogenous catecholamines. The area of cardiac histamine receptor subtypes is, however, extremely complex, not only because of the species variation but also because further variations appear to exist within any species, depending on the cardiac chamber being studied.\textsuperscript{13}

The present study was carried out to evaluate the effects of histamine on isolated neonatal rat ventricular myocytes in tissue culture. Although it is generally agreed\textsuperscript{19} that specific \( H_1 \) and \( H_2 \) receptors are absent from the adult rat heart, preliminary studies showed that the cultured myocytes exhibited a positive chronotropic response when exposed to histamine. Previous studies\textsuperscript{20} have indicated that in terms of their automaticity, these cells, in many ways, behave more like adult ventricular or Purkinje cells than like cells from the sino-atrial node. Since histamine enhances ventricular automaticity and may be associated with ventricular arrhythmias\textsuperscript{21} it was felt that these preliminary observations merited further investigation. The effects of histamine on the spontaneous contraction frequency of the cells were, therefore, evaluated in conjunction with those on the verapamil-sensitive Na\textsuperscript{+} influx and cAMP content of the cells. Further studies were carried out to determine the particular histamine receptor type in this preparation.

Materials and Methods

Myocardial Cell Cultures

Myocardial cell cultures were prepared from the hearts of 1- to 2-day-old Sprague-Dawley rats as previously described\textsuperscript{21} using the method of Harary and Farley,\textsuperscript{22} but incorporating modifications\textsuperscript{21,23} designed to improve the percent of myocardial cells present in the cultures.

Briefly, cultures were obtained from neonatal rat hearts, using only ventricular tissue, in order to obtain, as nearly as possible, a homogeneous cell population. The ventricles were cut into small pieces, approxi- mately 1 mm \( \times \) 1 mm and dissociated to single cells by repeated trypsinization. The resultant cell suspensions in growth medium (Minimal Essential Medium supplemented with 10% calf serum) were then cultured in a 95% air: 5% \( \text{CO}_2 \) atmosphere at 37° C. After 4 days in culture a satisfactory monolayer of approximately 1 \( \times \) 10\textsuperscript{5} cells per 6-cm diameter petri dish had formed. Prior to each study, randomly selected plates were examined under phase contrast microscopy to determine the percent of cells showing visible contractile shortening and to ensure that contractions were synchronous. Only those cultures in which at least 80% of the cells showed synchronous contractile shortening were used for the study.

Since the pH of the growth medium depends on a controlled atmosphere of 5% \( \text{CO}_2 \) in air, all studies were carried out after the cells had been equilibrated in a physiologic balanced salt solution (BSS) for a period of 3 hours as previously described.\textsuperscript{21} This solution (BSS) contained (mM) Na\textsuperscript{+}, 136.80; K\textsuperscript{+}, 5.35; Ca\textsuperscript{2+}, 2.25; Mg\textsuperscript{2+}, 1.03; Cl\textsuperscript{−}, 148.22; PO\textsubscript{4}\textsuperscript{3−}, 0.43; glucose, 11.10; plus calf serum, 5%; and, phenol red 0.0002% (pH 7.2). It has been shown that the contraction frequency and ionic content of the cells remained unchanged for at least 24 hours\textsuperscript{21} after incubation in BSS. All test drugs were freely water soluble and were dissolved in the BSS at 37° C. The desired final concentrations were obtained by serial dilutions in BSS. In this way the ionic composition of the drug-containing solutions was kept constant and any possible effect that could be ascribed to an alteration in the ionic milieu of the cells was avoided.

Measurement of Contraction Frequency

The contraction frequency of the cells in culture was measured by displaying the phase contrast microscope image of the cells, via a closed circuit television camera, on a 9-inch high resolution video monitor (total magnification 400 \( \times \)). A high-impedance photodiode, masked to a 1 mm slit aperture, was mounted on the TV screen with the slit perpendicular to the edge of a visibly contracting cell. The signal from the photodiode was passed through a low pass filter (0-10 Hz) to remove unwanted "noise" from the video monitor and displayed on an oscillographic recorder. Simultaneously the signal was recorded on a 2-channel direct-writing recorder to provide records of the contractile behavior of the cells. Contraction frequency was expressed as contractions per minute. Throughout the measurements, the Petri dish containing the cells, mounted on a microscope stage, was maintained at a constant 37° C using a Sage Air Curtain incubator (Sage Instruments Division, Orion Research Inc., Cambridge, Mass.), the thermistor probe of which was placed in the solution bathing the cells.

Measurement of Sodium Influx

Sodium influx was measured as previously described\textsuperscript{21} using \( ^{24}\text{Na} \) as a tracer. Briefly, the cells were exposed to \( ^{24}\text{Na} \), in BSS for 10 seconds during which time the cells were agitated gently on a controlled temperature warming plate at 37° C. Since the period of exposure to the isotope is short compared to the half-time of Na\textsuperscript{+} exchange (35 seconds),\textsuperscript{21} \( ^{24}\text{Na} \) efflux during this time is considered to be negligible.\textsuperscript{21,24} and hence no correction needs to be applied. Following the 10-second exposure to the isotope, the cells were quickly washed in a Na\textsuperscript{+}-K\textsuperscript{+} free isotonic Ca sorbitol solution\textsuperscript{21} at 0° C to remove the extracellular tracer. The \( ^{24}\text{Na} \) content of the cells was determined from the intracellular radioactivity and the specific activity of the soak solution.\textsuperscript{21} Total Na\textsuperscript{+} influx was calculated from the equation: M\textsubscript{Na} = \( \frac{\text{d}(C_i)}{\text{d}t} \cdot V/A \) where M\textsubscript{Na} is the influx (pmol/cm\textsuperscript{2}/sec), C\textsubscript{i} is the amount of ion entering the cells in time t and V/A is the volume to surface area of the cells. Previously reported values\textsuperscript{21} for cell surface area, cell volume, and volume of cell water were used for all calculations.
Separation of the Na\(^+\) influx into its verapamil-sensitive and verapamil-insensitive components was obtained from measurements carried out in the presence and absence of 10\(^{-5}\) M verapamil. This concentration has previously been shown to inhibit maximally the verapamil-sensitive component of the flux.\(^{20}\) Where the influx was being measured in the presence of verapamil the cells were pretreated with verapamil for two minutes to ensure complete blockade. The verapamil-sensitive component of the Na\(^+\) influx, under all conditions tested was calculated as the difference between the total Na\(^+\) influx and that remaining in the presence of 10\(^{-5}\) M verapamil.

**Measurement of Cyclic AMP**

The cAMP content of the cells was determined using a commercially available radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, Calif.), which is based on the competitive binding of [\(^3\)H]cAMP and endogenous cAMP to a limited amount of specific cAMP binding protein (anti-cAMP antibody).\(^{26}\) The detection limit of the assay is 0.05 pmol/50\(\mu\)g sample with a sensitivity of 0.025 pmol cAMP.

At the end of the appropriate treatment period, the incubating solution was quickly removed, and the cells were washed six times with ice-cold (0°C) phosphate-buffered saline (PBS). The cells in 1 ml of ice-cold PBS were gently scraped from the Petri dish, pipetted into a 12 \(\times\) 75 mm glass tube, and centrifuged at 4000 g for 10 minutes (0°C). Following centrifugation, the supernatant was discarded and the cell pellet was immediately frozen in acetone dry ice (\(\approx -65^\circ\)C) for at least 20 minutes. The cell pellet, homogenized in 1 ml of ice-cold 5\% trichloroacetic acid (0°C), was centrifuged at 4000 g for 20 minutes, the supernatant extracted 3 \(\times\) with acidified ethyl ether, and the aqueous phase evaporated to dryness. The residue was redissolved in 1 ml of 50 mM Tris-4 mM EDTA buffer. To assay for cAMP, 0.1 ml of the assay sample was mixed with 0.3 ml of Tris-EDTA buffer and 0.1 ml of [\(^3\)H]cAMP. cAMP-binding protein (0.1 ml) was then added, the solution thoroughly mixed and incubated for 90 minutes in an ice bath (0°C). Following the 90 minute incubation, charcoal-dextran (0.5 ml) was added to remove the free (unbound) cAMP, and the suspension was centrifuged at 2000 g for 20 minutes. The bound [\(^3\)H]cAMP, present in the supernatant, was determined using a liquid scintillation counter. The cAMP content of the sample was then obtained from a standard curve of bound/unbound cAMP, prepared in the same way as the samples but containing known amounts of cAMP.

In each experiment, parallel measurements of the protein content of the cells were carried out using the method of Lowry et al.\(^{27}\) The plates used for protein determination were treated in exactly the same way as those in which cAMP content were measured, permitting expression of cAMP results as pmol/mg protein. Two plates of cells were used for each cAMP and for each protein determination.

**Materials**

\(^{3}\)Na was obtained from New England Nuclear Corporation, Waltham, Mass. All isotope counting was carried out using a Packard Tri-Carb Liquid Scintillation Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Biologicals were from Gibco Laboratories, Grand Island, N.Y., and all chemicals used were of analytical grade. Diphenhydramine HCl and histamine HCl were from Sigma Chemical Co., St. Louis, Mo. Cimetidine HCl was obtained from Smith, Kline and French Laboratories, Philadelphia, Penn.; verapamil HCl from Knoll Pharmaceuticals, Whippny, N.J.; and [N-(cis-2-phenylcyclopentyl)azacyclotridecan-2-imine hydrochloride] (MDL 12330A) was generously supplied by Merrell Dow Pharmaceuticals, Inc., Cincinnati, Ohio.

**Statistical Methods**

Student's t tests were used to test for significant differences between paired and unpaired samples. Linear regression analysis was done by least squares fit. All results are expressed mean ± SEM.

**Results**

**Effect of Histamine on Contraction Frequency**

At 37°C, histamine, in the concentrations tested, produced a dose-dependent increase in spontaneous contraction frequency of the cells. This effect appeared to reach a maximum in the presence of 10\(^{-5}\) M histamine with further increases in histamine concentration leading to no further significant increments in contraction frequency (Figure 1). The spontaneous contraction frequency (contractions per minute) increased from a control of 121 ± 5 (mean ± SEM) to 153 ± 4, 181 ± 9, 212 ± 4, and 216 ± 6 in 10\(^{-4}\) M to 10\(^{-3}\) M histamine, respectively. Compared to control, the effect of each of the histamine concentrations tested was statistically significant (for each p < 0.001; n = 10). From the dose-response curve of the effect of histamine on the contraction frequency of these cells (Figure 1) it would appear that the automaticity of the preparation is sensitive to even low concentrations of histamine, half-maximal activation of the chronotropic effect occurring in the presence of 5 \(\times\) 10\(^{-5}\) M histamine.

The effect of histamine on the spontaneous contraction frequency of the cells did not develop immediately upon addition of histamine to the cells. The data presented in Figure 1 represent the steady-state response of the cells to treatment obtained after 30-minute exposure to the drug. The effect was time-dependent for each of the concentrations tested, but in every case had reached a maximum within 30 minutes. The time-dependence of the rate effect of 10\(^{-6}\) M histamine is shown in Figure 2.

**Effect of Histamine on Na\(^+\) Influx**

Like the effect on contraction frequency, the effect of histamine on the Na\(^+\) influx in the cells was time-dependent. Indeed, there was a close parallel between the increase in contraction frequency and that of the
Steady-state Na⁺ influx values, obtained following 30 minutes of histamine treatment, show a dose-dependence very similar to that of the contraction frequency (Figure 3). Total Na⁺ influx (pmol/cm²/sec) increased from a control value (mean ± SEM) of 26.1 ± 0.51 to 36.1 ± 1.03, 38.7 ± 1.47, 45.1 ± 1.53, and 47.2 ± 0.95 in the presence of 10⁻⁷ M, 10⁻⁶ M, 10⁻⁵ M, and 10⁻⁴ M histamine, respectively. For each concentration tested, the effect on Na⁺ influx, compared to control was significant (for each p < 0.001; n = 6).

With histamine treatment the mean verapamil-sensitive Na⁺ influx under steady-state conditions increased in a dose-dependent manner (Figure 3). The maximum effect occurred in the presence of 10⁻⁵ M to 10⁻⁴ M histamine (Figure 3), and both total and verapamil-sensitive Na⁺ influx were half-maximally stimulated in the presence of 5 × 10⁻⁷ M-10⁻⁶ M histamine.

**Effect of Specific H₁ and H₂ Antagonists on the Chronotropic Response to Histamine**

In an attempt to define, indirectly, the type of histamine receptor on the cultured neonatal rat myocardial cells responsible for both the chronotropic and Na⁺ influx effects of histamine, the preceding experiments were repeated in cells pretreated with either diphenhydramine (10⁻⁶ M to 10⁻⁴ M) or cimetidine (10⁻⁶ M to 10⁻⁴ M) for 30 minutes. Treatment with either agent alone did not affect either the contraction frequency or Na⁺ influx of the cells.

Pretreatment of the cells with diphenhydramine, in
concentrations up to and including $10^{-4}$ M, did not abolish or modify the response of the cells to histamine, in terms of contraction frequency (Figure 1) or Na$^+$ influx. On the other hand, pretreatment of the cells with increasing concentrations of cimetidine, from $10^{-6}$ M to $10^{-4}$ M, caused a progressive rightward shift of the dose-response curve of the effect of histamine on contraction frequency (Figure 1) suggestive of competitive inhibition. The highest concentration of cimetidine tested ($10^{-4}$ M) completely abolished the effect of all concentrations of histamine tested (Figure 1). Although $10^{-4}$ M cimetidine by itself, even when present for up to 30 minutes, had no effect on the contraction frequency or on the Na$^+$ influx it completely blocked the effect of any of the concentrations of histamine tested on both of those parameters (Figure 4). These results strongly suggest that the effects of histamine in this preparation are mediated via H$_2$ receptors.

**Effect of Histamine on Cellular cAMP Content**

The preceding results indicate that histamine increases both automaticity and verapamil-sensitive Na$^+$ influx in tissue cultured myocardial cells. Since the latter, like other slow-channel dependent functions, may be under the control of cAMP, further studies were undertaken to evaluate the effect of histamine on the cAMP content of the cells.

We found a time-dependent, dose-related increase in cellular cAMP (Figure 5) with each of the concentrations of histamine tested. The effect of histamine appeared to reach a maximum between 30 minutes and 60 minutes (Figure 5) with no further increase being elicited with further time exposure.

Having determined the time course of the effect of histamine on cellular cAMP content, we sought to determine the dose-response relationship between histamine and the cAMP content of cells treated for a 60-minute period. In response to histamine treatment, the increase in cAMP (Figure 6) followed a sigmoid dose-response relationship with half-maximal activation at around $10^{-3}$ M. Cellular cAMP (pmol/mg protein) rose from a mean ± SEM of 5.15 ± 0.16 to 6.51 ± 0.20, 7.98 ± 0.22, 9.36 ± 0.23, 15.00 ± 1.18, and 17.29 ± 1.36 in $10^{-7}$ M, $10^{-6}$ M, $10^{-5}$ M, $10^{-4}$ M, and $10^{-3}$ M histamine respectively. Compared to control cells the results in the presence of $\geq 10^{-6}$ M histamine are significant ($p < 0.001$; for each $n = 6-10$).

**Effect of Specific H$_1$ and H$_2$ Antagonists on cAMP Content in the Presence of Histamine**

Since the chronotropic and Na$^+$ influx effects of histamine were inhibited by cimetidine, but not di-
phenylephrine, and the verapamil-sensitive Na\(^+\) influx is probably influenced by cellular cAMP content we assessed the influence of histamine antagonists on the histamine-induced cAMP content elevations. Neither diphenhydramine or cimetidine in concentrations \(\leq 10^{-4}\) M, when applied alone had any effect on cellular cAMP content. Diphenhydramine did not influence the cAMP content in histamine-treated cells.

By contrast, pretreatment (30 minutes) of the cells with cimetidine, a specific H\(_2\) antagonist, in a concentration of \(10^{-6}\) M caused a rightward shift of the dose-response curve (Figure 6), suggesting competitive inhibition of the histamine effect by cimetidine. This observation lends further support to the fact that histamine effects are mediated via H\(_2\) receptors in this particular preparation.

**Effect of MDL 12330A on cAMP Response to Histamine Treatment**

Increases in cellular cAMP in response to histamine treatment could reflect either decreased cAMP degradation (inhibition of phosphodiesterase) or enhanced cAMP production (adenylate cyclase stimulation). To study these possibilities we measured the cAMP content in response to treatment with histamine, \(10^{-7}\) M to \(10^{-3}\) M, in the presence of MDL 12330A. This compound, [N-(cis-2-phenylcyclopentyl) azocyclotridecan-2-imine hydrochloride] has been shown to inhibit adenylate cyclase activity in cardiac tissues\(^18,20\) and more specifically histamine-stimulated adenylate cyclase activity.\(^26\)

In the absence of histamine, MDL 12330A did not affect the cAMP content of the cells. The compound did result in a small, but nonsignificant decrease in the spontaneous contraction frequency at concentrations between \(10^{-6}\) M and \(10^{-4}\) M. Pretreatment of the cells with \(10^{-4}\) M MDL 12330A, however, almost completely abolished the increase in cAMP seen in response to histamine (\(10^{-7}\) M to \(10^{-3}\) M) treatment (Figure 6). In addition, in the presence of \(10^{-6}\) M MDL 12330A, histamine produced no time- or dose-dependent increase in spontaneous contraction frequency.

**Relationship Between Verapamil-Sensitive Na\(^+\) Influx and Contraction Frequency in the Presence of Histamine**

Under all conditions tested, in the presence of histamine (\(10^{-7}\) M to \(10^{-4}\) M) we found (Figure 7) a close linear relationship between the verapamil-sensitive Na\(^+\) influx (pmol/cm\(^2\)/sec) and the spontaneous contraction frequency of the cells. This relationship could be described by a single linear regression, having the equation \(y = 4.97x + 29.13\) \((r = 0.92; p < 0.001)\), which is very similar to that previously derived\(^20\) for these cells from experiments in which extracellular Na\(^+\) was varied.

**Discussion**

The present study confirms the positive chronotropic effect of histamine in mammalian cardiac tissues that demonstrate intrinsic automaticity. The percent increase in spontaneous contraction frequency in this preparation, for each concentration of histamine tested, is very similar to the increase in sinus rate seen in the isolated hearts from guinea-pig,\(^10,30\) human fetus,\(^15\) cat,\(^18,31\) and monkey.\(^32,33\) Indeed in the present preparation half-maximal rate increases occur at a concentration of histamine (\(5 \times 10^{-7}\) M, Figure 1) that half-maximally activates the sinus node rate in the other species tested. The cells used in the present study, although derived from neonatal rat ventricular tissue, respond to histamine in a manner analogous to the sino-atrial or atrial cells of other species.

In view of the fact that the adult rat heart does not appear to contain either H\(_1\) or H\(_2\) receptors,\(^19\) the response of these neonatal myocytes to histamine may seem rather surprising. Since the effects of histamine are competitively inhibited by cimetidine the results further indicate that the effects seen are specific H\(_2\) mediated effects rather than a nonspecific response. Why these cells should differ from those of the adult rat is unclear, but perhaps this is a reflection of the fact that they represent a relatively immature myocardial cell type. This immaturity, including relative immaturity of enzyme systems, may be responsible for the relatively long time taken for histamine to elicit a maximal response. Despite these limitations, and especially since they do clearly respond to histamine, the advantages inherent\(^31\) in the preparation render it a suitable model in which to study some of the mechanisms underlying the effects of histamine on the heart.

The ionic currents underlying histamine-induced changes in automaticity have been incompletely defined. Only indirect evidence\(^16\) has implicated an inward Ca\(^{2+}\) current, since changing extracellular Ca\(^{2+}\) modified the effect of histamine on sino-atrial diastolic depolarization. In the present study, the effect of histamine appears to be related to an augmentation of the Na\(^+\) influx, especially the verapamil-sensitive component of the flux. Although it may be argued that the changes in Na\(^+\) influx result from, rather than determine, changes in contraction frequency, previous studies\(^30,34\) have shown that the contraction frequency of these cells is extremely and immediately sensitive to
changes in extracellular Na\(^+\).\(^{20,34}\) It should be pointed out that the relation between the verapamil-sensitive Na\(^+\) influx and contraction frequency defined in this study is almost identical to that previously defined under a variety of experimental conditions.\(^{20,35}\)

In the present studies no measurements of cellular electrical activity were made, and it was not possible to define specific membrane currents directly. Changes in the spontaneous contraction frequency of the cells however must be indicative of changes in automaticity of phase 4 diastolic depolarization in the cells of the monolayer. Further, it is probable that the rate of diastolic depolarization of all cells in the culture is similar because: a) of the tight electrical coupling that exists between adjacent cells; and b) the excitation wavefront changes from beat to beat with little or no change in the contraction frequency.\(^{36}\) It is felt that alterations in ion exchange associated with changes in automaticity reflect processes involving all of the cells of the monolayer.

It is generally accepted that spontaneous phase 4 depolarization, the phenomenon conferring automaticity, is attributable to a slow inward current.\(^9\) In sinoatrial tissue this appears to be carried by Ca\(^{2+}\)\(^{37,38}\) whereas in spontaneously firing Purkinje fibers\(^{39,40}\) and cultured chick and rat myocardial cells\(^{20,34,41}\) changing extracellular Ca\(^{2+}\) has little or no effect on automaticity. However, decreasing extracellular Na\(^+\) diminished or abolished pacemaker activity in both Purkinje fibers\(^40\) and cultured heart cells.\(^{20,34}\) It has been suggested that in Purkinje fibers and in ventricular myocardiun Na\(^+\) is responsible for the current conferring automaticity\(^42\) or that both Na\(^+\) and Ca\(^{2+}\) are involved.\(^{39,44}\) Thus, the cells in the present study behave more like ventricular or Purkinje cells than like sinoatrial tissue. Since histamine may enhance automaticity in Purkinje fibers, ventricular cells, and partially depolarized ventricular cells\(^4\) the present observation may have particular relevance to histamine-induced ventricular arrhythmias.

In these cells, as in other preparation,\(^1,3,4\) the enhanced automaticity in the presence of histamine appears to be related to an increase in cellular cAMP levels. Increases in cellular cAMP increase the slope of phase 4 depolarization\(^45,46\) presumably by increasing the number of available slow channels,\(^2\) thus augmenting the slow inward current regulating phase 4 diastolic depolarization. Evidence that cAMP is involved in the histamine effects in cultured heart cells comes from the experiments in which 10\(^{-6}\) M lactamimide (MDL 12330A) was used to inhibit histamine-stimulated adenylyl cyclase.\(^28,29\) In the presence of lactamimide none of the concentrations of histamine used increased cellular cAMP, and no increase in contraction frequency or Na\(^+\) influx was seen. It appears that the positive chronotropic effect of histamine in these cells is related to an increase in cAMP with subsequent augmentation of the verapamil-sensitive Na\(^+\) influx.

The effects of histamine in the adult rat do not appear to be mediated by either H\(_1\) or H\(_2\) receptors, but rather result from a histamine-induced release of endogenous catecholamines.\(^3\) Although such a possibility exists, it is unlikely that the effect of histamine in this tissue is dependent on catecholamine release. Very high concentrations of histamine (10\(^{-4}\) M to 10\(^{-2}\) M) were required to release catecholamines and cause a stimulatory effect in the adult rat.\(^19\) In addition,\(^19\) specific H\(_1\) and H\(_2\) antagonists had no effect on this response. In the present preparation, low concentrations of histamine had a significant chronotropic effect (ED\(_{50}\) = 5 x 10\(^{-7}\) M). The effects of histamine on the contraction frequency and Na\(^+\) influx were completely abolished by 10\(^{-4}\) M cimetidine, a specific H\(_2\) antagonist, while a lower concentration of cimetidine (10\(^{-6}\) M) produced a rightward shift of the cAMP-histamine dose response curve suggestive of competitive inhibition. A specific H\(_2\) antagonist, diphenhydramine, did not influence the effects of histamine on any of the parameters tested. It appears that the effect of histamine in cultured myocardial cells is mediated through H\(_2\) receptors, a finding similar to that in a wide variety of mammalian hearts.\(^1,3,4\) Since only H\(_2\) receptors are linked with adenylyl cyclase activity\(^37\) the increase in cAMP seen in the present study further strengthens the argument that H\(_2\) receptors are involved.

Compared to other tissues\(^1\) the responses of cultured heart cells to histamine are rather slow to develop. The reasons for this are not immediately apparent but may be a reflection of the neonatal origins of the tissue and possibly immature membrane enzyme systems. There is a close temporal relationship between the contraction frequency and Na\(^+\) influx on the one hand and cAMP levels on the other. This temporal relationship serves to support further the suggestion that cAMP is the essential second messenger in the chronotropic effects of histamine.

In summary, the positive chronotropic effects of histamine in cultured heart cells depends on a cAMP-mediated increase in the verapamil-sensitive Na\(^+\) influx. Further, these effects in turn are mediated via H\(_2\) receptors coupled to the adenylyl cyclase system.

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D McCall and C Y Lui

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