Histamine-Mediated Vasoconstriction and cAMP Levels in Coronary Arteries of the Isolated Rabbit Heart

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Abstract. The role of adenosine 3',5'-monophosphate (cAMP) in histamine-mediated vasomotion of coronary vascular smooth muscle was studied in the isolated perfused rabbit heart. In paired physiological studies, histamine-mediated vasoconstriction, as indicated by change in perfusion pressure, was inhibited by the presence of either theophylline, a phosphodiesterase inhibitor, or forskolin, an adenylate cyclase activator. The inhibitory effect of theophylline, but not of forskolin, was removed with cimetidine (H₂-receptor antagonist). In biochemical studies, coronary vessel cAMP was measured immediately after, and compared to, the vasomotor response to histamine alone and to histamine in the presence of forskolin, theophylline, diphenhydramine (H₁-receptor antagonist) or cimetidine. These studies showed that cAMP levels correlate inversely with the vasoconstrictor response to histamine, indicating that stimulation of H₁-receptors is associated with a reduction of cAMP and that H₂-receptors modify this reduction.

Introduction

The involvement of histamine with the adenylate cyclase system has been studied in a variety of tissues, the predominant findings being that the H₂-receptor is intimately linked to adenylate cyclase and that H₂-receptor stimulation enhances adenosine 3',5'-monophosphate (cAMP) levels [Bristow et al., 1982; Doua and Code, 1974; Johnson et al., 1979; McNeill, 1979; Sedor and Abboud, 1985; Vohra and Rautanen, 1982]. The role of H₁-receptors is uncertain. While most work has shown no interaction between H₁-receptors and adenylate cyclase, studies in brain have shown either potentiation [Al-Gadi and Hill, 1985; Schwartz et al., 1986a] or inhibition [Schwartz et al., 1986b] of adenylate cyclase by H₁-receptors.

Previous work from this laboratory has characterized the vasomotor response to histamine in the isolated rabbit heart as being an H₁-mediated vasoconstriction which is partially attenuated by H₂-receptors [Saari,
1986]. Because of the above-noted relationships between histamine and cAMP in other tissues, and because histamine-adenylate cyclase interactions have not been studied in coronary vascular smooth muscle, we thought it relevant to characterize further the effect of histamine in our preparation with respect to its effect on cAMP. Specifically, we wanted to determine if, and to what extent, histamine altered cAMP levels in rabbit coronary vessels and, secondly, to determine whether altered cAMP content could be correlated with the vasomotor response to histamine.

Methods

Experimental Preparation

The experimental preparation, presented previously [Saari, 1986], will be briefly reviewed. Male and female New Zealand albino rabbits, weighing 1.8–2.3 kg, were used for this study. They were anesthetized by injection of 2.0–3.0 cm$^3$ of sodium pentobarbital (5% Nembutal, Abbott$^1$) into the marginal ear vein. Heparin sodium (2,000 USP units, Sigma) solution was injected into the same vein to prevent clotting. The thorax was opened, the aorta cannulated, and the heart removed from the animal. Perfusion of the coronary vasculature was accomplished via retrograde perfusion of the aorta through the aortic cannula. Ventricular drainage was provided by short, flanged cannulas inserted into the ventricular walls. The perfusion cannula, with heart suspended from it, was attached to one arm of a plastic ‘T’.

Two platinum electrodes were inserted into the heart to provide electrical pacing. One was inserted in the ventricle near the apex and the other in the right ventricle near the base. Stimulation of 120 beats/min at 3–5 V was achieved with a Grass S-44 stimulator and an SIU5 stimulus isolation unit.

A Masterflex constant flow pump drew solutions in parallel from 4 reservoirs through tubing in temperature baths kept at 28°C. From the temperature baths, the parallel lines passed through parallel switches which allowed for the selection of one solution to perfuse the heart while the others were recycled. All solutions were oxygenated in their respective reservoirs with a humidified gas mixture of 95% O$_2$ and 5% CO$_2$. Flow rates were in the range of 45–50 ml/min. A schematic of the perfusion apparatus has been published earlier [Saari, 1986].

Perfusion pressure was monitored a few centimeters upstream from the heart by a Statham P23AC pressure transducer. The pressure was recorded on a Gould 2400 recorder.

Perfusion Solutions

The control Ringer-Tyrode perfusion fluid had the following composition (mmol/l): NaCl 137, KCl 2.68, CaCl$_2$·2H$_2$O 1.82, MgSO$_4$·7H$_2$O 1.25, glucose 5.55, NaHCO$_3$ 11.9. Experimental solutions were made up by freshly dissolving the appropriate drugs in the above control solution. Drugs used were histamine dihydrochloride, diphenhydramine hydrochloride, cimetidine, theophylline, and forskolin (all from Sigma, St. Louis, Mo.).

Protops

Physiological Studies. Because there is considerable interanimal variability in the degree of vasoconstrictor response to histamine, it was important to design experiments which yielded paired data for drug effects on the histamine response. Also, the tachyphylactic nature of the histamine response had to be taken into account for each animal [Saari, 1986].

The general protocol was to make four successive perfusions with histamine (1.1 × 10$^{-4}$ mol/l), each of 3.5 min duration, separated by 12-min recovery periods. When the effect of a drug on the histamine response was to be studied, it was applied prior to (as a pretreatment) and during the second histamine application. The change in perfusion pressure (ΔP) during this second run was then compared to a calculated or predicted response made by interpolation between the first and third histamine perfusions. The interpolation of a predicted response assumed that an exponential decay would have occurred in the absence of the drug, as has been reported previously [Saari, 1986].

$^1$ Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.
In an earlier work, the above protocol was used to assess the effect of $H_1$- and $H_2$-receptor antagonists, diphenhydramine and cimetidine, respectively, on the vasoconstrictor response to histamine [Saari, 1986]. In the present work, the protocol was used to determine the effects of forskolin (1.0 × 10^-6 mol/l), an adenylylate cyclase potentiator [Seamon and Daly, 1981], and theophylline (1.1 × 10^-5 mol/l), a phosphodiesterase inhibitor [Rall, 1985] on the histamine response. Pilot studies showed these substance concentrations to have no effect of their own, therefore allowing the monitoring of their possible potentiation of histamine effects. The protocols for these substances differed only in that the second-run pretreatment period for theophylline was 6 min while that for forskolin was 2 min.

In subsequent experiments the above protocols were revised by application of cimetidine (4.0 × 10^-5 mol/l) on all four histamine applications to determine whether either theophylline or forskolin required an $H_2$-receptor for its activity.

cAMP Assay. cAMP was measured in 6 different treatment groups of 4 hearts each. For each heart the assay was made at a time comparable to when the above described physiological comparisons were made for forskolin and theophylline (as were prior ones for diphenhydramine and cimetidine [Saari, 1986]); that time was 19 min, the time following the second histamine run. The 6 treatment groups were as follows: a control group, in which cAMP assay was done after 19 min of Ringer-Tyrode perfusion; a histamine group, in which assay was done after the second application of histamine (1.1 × 10^-4 mol/l) alone; 4 treatment groups in which the effect of histamine during the second run was modified by diphenhydramine (3.4 × 10^-7 mol/l), cimetidine (4.0 × 10^-5 mol/l), forskolin (1.0 × 10^-6 mol/l) or theophylline (1.1 × 10^-5 mol/l) and assay done immediately thereafter. Note that the coronary perfusion pressures immediately prior to the assays were monitored for subsequent correlation with the assay results.

The assay procedure for cAMP was begun at the 19-min point by perfusing each heart with an ice-cold, nonoxygenated, Ringer-Tyrode solution which contained theophylline to prevent cAMP metabolism. During this perfusion the heart was also placed in an ice-cold bath of the theophylline solution to assure maximal cooling and exposure to the phosphodiesterase inhibitor. After the 2-min theophylline perfusion and cooling, the heart was kept in the ice-cold bath while the superficial coronary arteries were removed as rapidly as possible (about 5 min to excise all tissues) and immediately frozen in liquid N$_2$ and stored at -70°C. The frozen arteries were lyophilized and weighed to determine a dry tissue weight. We used an extraction procedure described by Kroeger [1979].

The dry tissue was placed into test tubes containing 0.5 ml aliquots of hot (100 °C) buffer solution containing 4.4 mol/l EDTA and 50 mmol/l Tris-HCl with pH adjusted to 7.8 at 23 °C. After 5 min at 100 °C, to inactivate enzymes, the tubes were transferred to a shaking ice-water bath for 2 h. After extraction, the cAMP was measured in the supernatant by a competitive protein binding assay using a cAMP kit TRK 432 obtained from Amersham (Arlington Heights, Ill.). The method employed was essentially that described by Gilman [1970], the only difference being that charcoal rather than Millipore filters was used to separate bound from free cAMP.

**Statistical Analysis**

In the physiological studies, when observed results were compared to expected, the t test for paired data was used [Sokal and Rohlf, 1981]. The cAMP concentrations from the assay of the 6 groups of hearts were compared using one-way analysis of variance and Tukey's studentized range test for comparison of means [Kleinbaum and Kupper, 1978].

For those data for which correlations were desired (between cAMP concentration and coronary pressure), the significance of each correlation was determined by the t test in which t is equal to $r\sqrt{(n-2)/(1-r^2)}$, where $r$ is the correlation coefficient and $n$ is the number of data pairs [Sokal and Rohlf, 1981]. Minimal significance in all cases was regarded as p < 0.05.

**Results**

**Physiological Studies**

**Response to Histamine.** Representative recorder tracings of the response of the rabbit heart to an infusion of histamine have been illustrated previously. The variable of interest for these physiological studies is the difference between the maximum developed coronary perfusion pressure under histamine
(range 92–185 mm Hg) and the initial or baseline pressure prior to histamine application (range 49–100 mm Hg). Because the pressure response varied so greatly between hearts, for purposes of illustration the pressure change during each application of histamine was represented as a percentage of that occurring during the first application of histamine. When this was done for several hearts, each of which underwent four successive infusions of histamine, and the pressure responses for each run were averaged, a plot such as figure 1a was the result. Prior work has shown this pressure response to be caused by a predominant H1-receptor-mediated vasoconstriction modified by a H2-receptor-mediated inhibition, with H2-receptors apparently becoming more effective with successive histamine applications. These conclusions are based on figure 1b, in which H2-receptors were blocked by cimetidine on every run (note the enhanced response and reduced tachyphylaxis). The data in figure 1a,b represent control studies for the present work, but were redrawn from prior work [Saari, 1986].

**Effect of Theophylline on Histamine Response.** A representative recording of the response of the rabbit heart to a coronary infusion of histamine (1.1 × 10⁻⁴ in the presence of theophylline (1.1 × 10⁻⁵ mol/l) is shown in figure 2a; the upper tracing illustrates the typically low perfusion pressure increase observed. The degree to which the histamine-mediated vasoconstrictor response was depressed by theophylline is illustrated in figure 1c which shows the result of theophylline infusion prior to and during the second of four successive histamine infusions. The actual response during the second run is compared to one calculated (see Methods) by assuming that without theophylline the de-

**Fig. 1.** Vasoconstrictor responses (changes in coronary perfusion pressure) for four successive infusions of histamine for each of n hearts (see Methods for protocols). Response is calculated as the percentage of initial pressure change and runs for all hearts are averaged. Conditions for each panel: a Four infusions of histamine alone (n = 6); b four infusions of histamine, all in the presence of cimetidine (n = 7); c as for a but with theophylline present prior to and during the second histamine infusion (n = 6); d as for b, but with theophylline present prior to and during the second histamine infusion; e as for a, but with forskolin present prior to and during second histamine infusion (n = 5); f as for b, but with forskolin present prior to and during second histamine infusion (n = 4). Open bars in e–f represent calculated values based on the assumption that without theophylline or forskolin the response would have been exponential (see Methods).
Fig. 2. Representative recordings of coronary perfusion pressure, contractile force and first derivative of contractile force (dF/dt) using the following perfusion protocols: a Switch from theophylline to theophylline-histamine (†) and back to control solution (↓); b switch from cimetidine-theophylline to cimetidine-theophylline-histamine (†) and back to cimetidine (↓); c switch from forskolin to forskolin-histamine (†) and back to control (↓); d switch from cimetidine-forskolin to cimetidine-forskolin-histamine (†) and back to cimetidine (↓).
cay of response would have been exponential, as indicated from studies with control hearts (fig. 1a) [Saari, 1986]. The pressure change (vasoconstriction) under theophylline was lower ($p < 0.001$) than that predicted in the absence of theophylline.

The diminished vasoconstrictor response suggests that theophylline may either reduce the $H_1$-receptor effect (vasoconstriction) or enhance the $H_2$-receptor effect (inhibition). To investigate these possibilities, the above experiment was repeated in the presence of cimetidine, the $H_2$-receptor antagonist. A representative recording of the response to coronary infusion of histamine and theophylline in the presence of cimetidine ($4.0 \times 10^{-5}$ mol/l) is shown in figure 2b. The upper tracing illustrates the typically high-pressure response observed in comparison to that seen in figure 2a. The result of infusing theophylline prior to and during the second infusion of histamine with cimetidine present is illustrated in figure 1d. Cimetidine clearly eliminated the depression of the histamine response caused by theophylline, thereby indicating that theophylline acts by enhancing the $H_2$-mediated inhibition.

Figure 2 shows that, in addition to measuring the vasoconstrictor response to histamine, we also measured the corresponding responses in cardiac force (middle traces) and the first derivative of force (bottom traces), the latter being regarded as a measure of contractility [Beckett, 1970; Döring and Dehnert, 1987]. This was useful because the positive inotropic effect of histamine is known to be an $H_2$-mediated effect [Coruzzi et al., 1979; Sakai, 1980; Saari, 1986] and has been used to index the degree of $H_2$ antagonism [Saari, 1986]. Comparison of the force traces in figure 2a and b illustrates that cimetidine blocks the force response of histamine even in the presence of theophylline; this response parallels that seen in the coronary vessels and thus corroborates our suggested relationship between theophylline and $H_2$-receptors in coronary vessels. Also, because $H_2$-mediated inotropy in cardiac muscle has been associated with altered cAMP [Eckel et al., 1982], our use of the cardiac muscle response to corroborate interpretation of vascular smooth muscle data is further justified.

Effect of Forskolin on Histamine Response. A representative recording of the response of the rabbit heart to an infusion of histamine ($1.1 \times 10^{-4}$ mol/l) in the presence of forskolin ($1.0 \times 10^{-6}$ mol/l) is shown in figure 2c. The effect on the vasoconstrictor response of infusing forskolin during the second of four infusions of histamine is illustrated in figure 1e. The vasoconstrictor response was significantly ($p < 0.01$) depressed by forskolin, as indicated by comparison of actual to predicted response (see Methods).

Again, as for theophylline, the argument could be made that forskolin either inhibited the vasoconstrictor ($H_1$) response or potentiated the inhibitory ($H_2$) response. Cimetidine was used again to test these possibilities. A representative recording of the response to an infusion of histamine in the presence of forskolin and cimetidine is shown in figure 2d. The effect of cimetidine on the histamine response when forskolin was present during the second of four histamine infusions is shown in figure 1f. It is evident that cimetidine did not eliminate the depression of the histamine response by forskolin, suggesting, on first interpretation, that the vascular $H_2$-receptor is not associated with cAMP production. However, inspection of the contractility tracings in figure 2d
reveals that cimetidine in the presence of forskolin had no effect on histamine-induced contractility enhancement, a known H₂-mediated response. Because cimetidine at this concentration (4.0 × 10⁻⁵ mol/l) blocked the effect of histamine on contractility in the absence of forskolin [Saari, 1986], we suspect that forskolin interfered with cimetidine activity and may therefore be inappropriate for illustrating a relationship between H₂-receptors and adenyl cyclase.

*cAMP Assay*

The results of the assay of the coronary arteries for cAMP are presented in table 1.

When comparing control vessels to vessels of other treatment protocols, either histamine infusion or infusion with histamine and cimetidine (H₁-receptors stimulated) significantly depressed cAMP levels. No other treatment protocol resulted in cAMP concentrations significantly different from the control values.

When comparing histamine-treated vessels to vessels of other treatment protocols, most treatments significantly altered cAMP levels. In experiments in which we used selective H₁- and H₂-receptor antagonists, the use of diphenhydramine (H₁-blocker) increased cAMP concentrations relative to those for histamine alone, whereas the use of cimetidine (H₂-blocker) showed a nonsignificant trend toward reduced cAMP concentrations relative to those for histamine alone. In experiments in which we attempted to alter cAMP levels by nonreceptor mechanisms, theophylline, the phosphodiesterase inhibitor, and forskolin, the adenylate cyclase potentiator, both showed higher cAMP concentrations than those with histamine alone, thus corroborating their use for such purposes in this preparation.

![Fig. 3. Increase of coronary perfusion pressure versus cAMP concentration with various treatment conditions (see Methods). × = Control, no pressure change; ● = histamine treatment; ○ = histamine in the presence of cimetidine; □ = histamine and diphenhydramine; ■ = histamine and theophylline; ▲ = histamine and forskolin.](image)

**Table 1.** Coronary artery cAMP concentrations (means ± SD) with various treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>cAMP, pmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.37 ± 0.02a</td>
</tr>
<tr>
<td>Histamine (Hist)</td>
<td>0.30 ± 0.03b</td>
</tr>
<tr>
<td>Hist-diphenhydramine</td>
<td>0.39 ± 0.01a</td>
</tr>
<tr>
<td>Hist-cimetidine</td>
<td>0.25 ± 0.03b</td>
</tr>
<tr>
<td>Hist-theophylline</td>
<td>0.39 ± 0.03a</td>
</tr>
<tr>
<td>Hist-forskolin</td>
<td>0.40 ± 0.03a</td>
</tr>
</tbody>
</table>

a, b Values with different superscripts are significantly different (p < 0.05).

* cAMP-Vasoactivity Correlation. The concentration of cAMP in coronary vessels following each treatment protocol was plotted against the change in coronary pressure occurring just before the vessels were removed for assay (fig. 3). The correlation coefficient r = -0.82 for the two variables was highly
significant \((p < 0.001)\). Though in the above correlation we plotted changes in pressure to normalize for the variability in baseline pressure, plotting absolute pressures at the peak of the response also resulted in a highly significant correlation with cAMP concentration \((r = -0.68, p < 0.01)\).

**Discussion and Conclusions**

**Physiological Studies**

In our physiological studies, two substances, theophylline and forskolin, at concentrations which have no physiological effect of their own, inhibited the vasoconstrictor response of coronary vessels to histamine (fig. 1c, d). They were both used for their purported ability to raise, by different mechanisms [Rall, 1985; Seamon and Daly, 1981], tissue concentrations of cAMP. That they raised cAMP concentrations in the presence of histamine, relative to that produced by histamine alone, was supported by the results of the cAMP assay (table 1). Thus, we associate the elevated cAMP concentrations with the relative vasodilation produced by these substances.

In the case of theophylline, other possible mechanisms of action must be considered. Theophylline, in addition to phosphodiesterase inhibition, has been associated with translocation of intracellular calcium as well as with antagonism of adenosine activity; we feel that both of these alternative mechanisms may be largely discounted. In contractile tissues where calcium translocation induced by methylxanthines has occurred [Bianchi, 1968; Blinks et al., 1972], the physiological result was a contraction, not the relaxation which occurred in our preparation. Regarding potential adenosine antagonism, adenosine is known to cause coronary vasodilation [Rall, 1985] and has done so in our preparation (unpublished observations). If theophylline were antagonizing endogenous adenosine activity in this preparation we would expect relative vasoconstriction, not vasodilation. Though we cannot rule out a degree of adenosine antagonism, we feel that the elevated cAMP concentration is the predominant effect and is likely responsible for the relative vasodilation caused by theophylline. Supporting this conclusion is the fact that forskolin raised cAMP concentration by a different mechanism, adenylate cyclase potentiation, yet produced the same physiologic response.

In a variety of tissues, elevated cAMP concentrations have been associated with \(H_2\)-receptor stimulation (see Introduction). The fact that cimetidine, an \(H_2\)-receptor antagonist, blocked the inhibitory response of theophylline (fig. 1d), supports the notion that theophylline potentiated \(H_2\)-receptor-mediated cAMP production, thereby inhibiting vasoconstriction. We anticipated that cimetidine would also block the forskolin response. That it did not (fig. 1f) might at first suggest that the \(H_2\)-receptor is not associated with adenylate cyclase. However, because cimetidine, at a concentration which blocked histamine \(H_2\)-mediated inotropic in cardiac muscle [Saari, 1986]), could not do so in the presence of forskolin (fig. 2d) suggests that forskolin may not be the proper tool to investigate \(H_2\)-mediated mechanisms. We believe that forskolin may have either (1) altered the \(H_2\)-receptor and hence the binding of a rather selective \(H_2\)-antagonist or (2) sensitized hormonal stimulation of adenylate cyclase to the point that concentrations of antagonist which usually block receptor-mediated enzyme activity were insufficient at the
heightened level of activity. We did not pursue these hypotheses experimentally, but believe them to be a promising direction for future work.

Our conclusion from the above work, based largely on the theophylline findings and in spite of the forskolin findings (for reasons stated), is that H₂-receptors inhibited vasoconstriction in our preparation by increasing cAMP production.

**cAMP Assay and Correlation with Vasomotor Response**

Among the major findings of this study is that histamine stimulation of H₁-receptors is associated with a reduction in coronary vessel cAMP. Further, augmentation of the H₂-receptor component of the response, by H₁-receptor antagonism, by phosphodiesterase inhibition or by adenylate cyclase potentiation, increases cAMP concentrations above those occurring in the presence of histamine alone (table 1). Finally, the cAMP content for a given treatment correlates well with the corresponding degree of vasoconstrictor response to histamine (fig. 3).

An unexpected finding, in light of other work on H₂-receptors, was the inability of H₂-receptor stimulation to increase cAMP content beyond control levels, even in the presence of H₁-antagonism, phosphodiesterase inhibition or adenylate cyclase potentiation. We believe this inability to augment control cAMP concentration is consistent with our inability to cause overt vasodilation with histamine in our isolated heart preparation even with H₁-receptor antagonism. Apparently the H₂-receptor in our preparation simply modifies the predominant H₁-mediated vasoconstriction [Saari, 1986]; similarly, from this study, H₂-receptors apparently only modify existing levels of cAMP. This may be dependent on environment, that is, whatever conditions exist in our system (temperature, substrate availability) to prevent overt vasodilation (as opposed to inhibition of vasoconstriction) also appear to prevent cAMP production beyond control levels. This line of reasoning seems to support, although indirectly, our contention that the two variables are related.

A possible mechanism for the H₁-inhibition of cAMP production is suggested by recent experiments with the β-adrenergic receptor system (an activator of adenylate cyclase). Histamine, through an H₁-receptor mechanism, apparently uncouples the β-receptor from the guanyl nucleotide binding protein [Sastre et al., 1987]. A similar H₁-mediated inhibition of H₂-receptors, which have similarities to β-receptors [Mantelli et al., 1982], is a distinct possibility. In addition, the fact that H₁-receptor stimulation decreases cAMP from control levels suggests that H₁-receptors inhibit endogenously activated β (or other) receptors.

Another potential, although speculative, mechanism for H₁-mediated inhibition of cAMP production involves the H₁-mediated smooth muscle activation pathway whereby intracellular calcium is elevated via myoinositol triphosphate production [Berridge, 1984; Carswell et al., 1985; Mitchell, 1975; Villalobos-Molina and Garcia-Sainz, 1983]. With activation of this mechanism, the resulting use of ATP for phosphorylation of myosin light chains may sufficiently reduce ATP availability for use as a substrate by adenylate cyclase. In this case reduced cAMP levels caused by H₁-activation would be an indirect effect, but could nevertheless serve as positive feedback enhancement of the myoinositol triphosphate-induced contraction.
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