Histamine inhibits atrial myocytic ANP release via H₂ receptor-cAMP-protein kinase signaling

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Roles for the cholinergic and adrenergic systems have been reported to have variable effects on ANP release (32). Effects of peptide hormones on ANP release have also been observed (12).

Previously, it was shown that histamine H₁ and H₂ receptors are expressed in the atrium (18). Also, it was known that the heart contains as much as 3 μg histamine/g (13). Effects of histamine on the regulation of cardiac function are species specific and also tissue specific, even in the same animal. Histamine has been known to increase force of contraction in rabbit and human atria via activation of adenylyl cyclase, and to increase cAMP production (18, 28, 35). Both the positive inotropic effect and increase in cAMP production were blocked by cimetidine but not mepyramine, which indicates the effects are related to the activation of histamine H₂ receptor (18). It was shown in the rabbit atria that the increase in contractility and cAMP production by histamine is caused exclusively by an activation of H₂ receptor (17). Increase in intracellular cAMP production may increase Ca²⁺ influx via cAMP-dependent protein kinase A (PKA), which in turn may result in an increase in contractility (14, 31). It was shown that histamine-induced increase in Ca²⁺ current is blocked by H₂-receptor antagonist cimetidine and PKA inhibitor RP-adenosine 3′,5′-cyclic monophosphorothioate (RP-cAMP[S]) (19).

There are diverse reports on the effects of cAMP and Ca²⁺ in the regulation of ANP release. Increase in cAMP production by adenylyl cyclase activation with forskolin decreased atrial ANP release in perfused atria and hearts (7, 34). In contrast, it has also been shown that cAMP-elevating agents and cAMP analogs increase ANP secretion in cultured cardiac myocytes (5), sliced atria (1), isolated atria (36), and perfused heart (33). Diverse effects of Ca²⁺ on ANP release have also been reported: positive role of Ca²⁺ (33, 36) and negative inhibitory role of Ca²⁺ (9, 21, 23, 34). Hence, the present understanding of cAMP- or Ca²⁺-dependent regulation of ANP release is controversial.

In the present study, we propose the hypothesis that histamine may regulate atrial myocytic ANP release...
because 1) ANP stimulates histamine release from mast cells (30) and thus a reciprocal regulation may exist, 2) histamine elevates atrial cAMP levels (17, 18, 28, 35), and 3) evidence has been provided to suggest that an increase in cAMP modulates atrial ANP release (7). Because it was shown that acute stress induces cardiac mast cell activation and histamine release (20) and that histamine is involved in the coronary vasoconstriction (15), it is also possible to hypothesize that the ability of histamine to induce coronary vasoconstriction may, at least in part, be related to its ability to modulate ANP release. The purpose of the present study was to define the effect of histamine on atrial ANP release and to elaborate the receptors related to that and to disclose the postreceptor intracellular signaling.

![Fig. 1. Protocols for present experiments. Solid bars show the period of sample collections to be compared with control (Cont) or each other. For time control, corresponding period was compared. Atria were paced at 1.3 Hz. Cime, cimetidine; Tripro, triprolidine; Hist, histamine; Nife, nifedipine; Stauro, staurosporine; KT, KT5720; RP, RP-cAMP[S].]
METHODS

Beating perfused atrial preparation. New Zealand White rabbits were used. An isolated perfused atrial preparation was prepared by the method described previously (3, 6), which allowed atrial pacing and measurements of changes in atrial volume during contraction (stroke volume), pulse pressure, transmural extracellular fluid (ECF) translocation, cAMP efflux, and ANP secretion. The atrium was perfused with HEPES-buffered solution by means of a peristaltic pump (1 ml/min).

Experimental protocols. The atria were perfused for 60 min to stabilize ANP secretion. The atria were paced at 1.3 Hz. [3H]Inulin was introduced to the pericardial fluid 20 min before the start of the sample collection (3). The perfusate was collected at 2-min intervals at 4°C for analyses. Experiments were carried out by using six groups of atria to define receptor specificity (Fig. 1). Control cycles (two 12-min periods) were followed by histamine (group 1, 10 μM, n = 10; see Figs. 5A and 6) for 3 cycles (36 min). The effects were evaluated after two cycles (24 min) of administration of the agent. For the time-matched control, vehicle was introduced and values obtained during the periods corresponding to the control and experimental observations were compared (group 19, n = 10; see Fig. 6). One cycle (12 min) of infusion of histamine receptor subtype-selective antagonist was followed by an administration of histamine or vehicle in the presence of the prior agent. The following antagonists were used: 1) histamine H2-receptor antagonist cimetidine (group 2, 10 μM, n = 10, see Figs. 5A and 6; and group 3, n = 5, see Fig. 6); and 2) histamine H1-receptor antagonist triprolidine (group 4, 10 μM, n = 10, see Figs. 5B and 6; and group 5, n = 7, see Fig. 6). In another series of experiments, to define the effect of cell-permeable cAMP analog 8-Br-cAMP, control cycles (two 12-min periods) were followed by 8-Br-cAMP at 0.3 mM (group 6, n = 7; see Fig. 8), 0.6 mM (group 7, n = 9; see Figs. 7A and 8), 1.0 mM (group 8, n = 5; see Fig. 8), and vehicle (group 9, n = 6; see Fig. 8). In another series of experiments, to define involvement of L-type Ca2+ channels or protein kinases, three cycles (36 min) of infusion of inhibitors was followed by an infusion of histamine or vehicle in

Fig. 2. A: effects of Hist (10.0 μM) on ANP secretion (Ao), extracellular fluid (ECF) translocation (Ab), atrial natriuretic peptide (ANP) concentration (Ac), cAMP efflux (Ad), cAMP concentration (Ae), atrial stroke volume (Af), and pulse pressure (Ag) in perfused beating rabbit atria (1.3 Hz; n = 10). B: time-matched control for the same parameters (Ba–Bg) were stable during the period corresponding to Hist infusion (n = 10). Values are means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001 vs. Cont period.
the presence of the prior agent. Control cycles (four 12-min periods) were followed by histamine (group 10, 1.0 μM, n = 9, Fig. 4; group 11, 10 μM, n = 10, Figs. 2A–4, 11, 13, and 15; group 12, 30 μM, n = 9, Fig. 4) for 3 cycles (36 min). The following inhibitors were used: 1) L-type Ca\(^{2+}\)-channel inhibitor nifedipine (group 13, 1 μM, n = 7, Fig. 9A; and group 14, n = 6, Fig. 9B); 2) PKA inhibitors including KT5720 at 3 μM (group 19, n = 4, Fig. 13), 6 μM (group 20, n = 9, Figs. 12A, 13, 15; group 22, n = 5, Figs. 12B, 13 and 15), and 10 μM (group 21, n = 8, Fig. 13), and RP-cAMP[S] (group 23, RP-cAMP[S], 125 μM, n = 4, Figs. 14A and 15); and group 24, n = 3, Figs. 14B and 15); 3) the nonspecific protein kinase inhibitor staurosporine at 0.01 μM (group 15, n = 5, Fig. 11), 0.03 μM (group 16, n = 8, Fig. 11), and 0.1 μM (group 17, n = 7, Figs. 10A and 11; and group 18, n = 6, Figs. 10B and 11). The effects of histamine in the presence of modulating agent or vehicle were compared with the periods before and the third 12-min period after agonist. For the time-matched (group 25, n = 10, Figs. 2B, 4, 13, and 15) or modulating-agent control, values obtained during the periods correspond-
and, therefore, indicates the rate of myocytic release of ANP into the surrounding paracellular space (2, 3). It was calculated as ANP released \((\text{in } \mu\text{g}/\text{hr})/1000\text{ min}\) divided by immunoreactive ANP \((\text{in pg}/100\text{ min})/\text{g}\) of atrial tissue. Most of the ANP secreted is processed ANP (3).

Radioimmunoassay of cAMP. cAMP was measured by equilibrated radioimmunoassay (6, 7). Briefly, standards and samples were taken up in a final volume of 100 μl of 50 mM sodium acetate buffer (pH 4.8) containing theophylline (8 mM), and then 100 μl of diluted cAMP antiserum (Calbiochem-Novabiochem, San Diego, CA) and iodinated 2'-O-monomosuccinyl-adenosine 3',5'-cyclic monophosphate tyrosyl methyl ester \((^1\text{H})\text{cAMP-TME; 10,000 cpm/100 μl}\) were added and incubated for 24 h at 4°C. For the acetylation reaction, 5 μl of a mixture of acetic anhydride and triethylamine was added to the assay tube before the addition of antiserum and tracer. The bound form was separated from the free form by charcoal suspension. \(^1\text{H}\text{cAMP-TME was prepared as described previously (6, 38). Radioimmunoassay for cAMP was done on the day of experiments, and all samples from one experiment were analyzed in a single assay. Nonspecific binding was <2.0%. The 50% intercept was at 16.5 ± 0.79 fmol/tube (n = 10). The intra- and interassay coefficients of variation were 5.0 (n = 10) and 9.6% (n = 10), respectively. Nonspecific value of the buffer solution was subtracted from the value of atrial perfusate. The amount of cAMP efflux was expressed as pmol cAMP per minute per gram of atrial tissue. The molar concentration of cAMP efflux in terms of ECF translocation, which may reflect the concentration of cAMP in the interstitial fluid (2, 3, 6), was calculated as:

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\frac{\text{cAMP efflux (in pmol/minute/g)}}{\text{ECF translocated (in μL/minute/g)}}
\]

For the preparation of perfusates, 100 μl of the perfusate were treated with trichloroacetic acid (900 μl) for a final concentration of 6% for 15 min at room temperature and were

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**Fig. 5.** Effects of cimetidine (Cime; 10 μM; n = 10; A) and triprolidine (Tripro; 10 μM; n = 10; B) on Hist-induced decrease in ANP secretion (Ac and Bc), ECF translocation (Ab and Bb), ANP concentration (Ac and Bc), cAMP efflux (Ad and Bd), cAMP concentration (Ac and Bc), atrial stroke volume (Af and Bf), and pulse pressure (Ag and Bg) in perfused beating rabbit atria (1.3 Hz). Values are means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

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**Fig. 6.** Comparison of effects of Cime and Tripro on Hist-induced changes in ANP concentration and atrial dynamics. Values are difference in percent changes at the last 2 periods over the mean of the 2 periods before Hist. Values are means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001.**
centrifuged at 4°C. The supernatant (500 μl) was transferred to a polypropylene tube, extracted with water-saturated ether (1 ml) three times, and then dried by using a SpeedVac concentrator (Savant, Farmingdale, NY). The dried samples were resuspended with sodium acetate buffer.

Statistical analysis. Significant difference was compared by using repeated-measures ANOVA followed by Bonferroni’s multiple-comparison test (Figs. 2, 5, 7, 9, 10, 12, and 14). Student’s t-test for unpaired data (Figs. 4, 6, 8, 11, 13, and 15) was also applied. Correlation coefficients were determined with the use of linear regression analysis. Statistical significance was defined as P < 0.05. The results are given as means ± SE.

RESULTS

Histamine decreases atrial ANP release in a concentration-dependent manner. ANP secretion, ECF translocation, and the concentration of ANP in perfusate in terms of the ECF translocation, which reflects the rate of atrial myocytic ANP release, were stable during the control periods (Fig. 2, Aa–Ac and Ba–Bc). cAMP efflux, the concentration of cAMP in perfusate in terms of the ECF translocation and atrial dynamics, atrial stroke volume, and pulse pressure were also steady and stable (Fig. 2, Ad–Ag and Bd–Bg). Histamine (10 μM) decreased the secretion of ANP and the concentration of ANP (ANP release) (Fig. 2, Aa and Ac). Histamine increased atrial dynamics concomitantly with an increase in cAMP efflux and the concentration of cAMP (Fig. 2, Ad–Ag). Histamine-induced decrease in ANP release and increase in cAMP concentration continuously progressed up to ~18 min and was then maintained thereafter (Figs. 2, Aa and Ac, and 3A). However, histamine-induced increase in atrial dynamics showed a peak response and waned (Fig. 2, Af and Ag). Decrease in atrial myocytic ANP release by histamine was coincident with an increase in cAMP production (Fig. 3A) and was a function of atrial cAMP production (Fig. 3B). Because the binding of histamine to H₂ receptor triggers an early specific response characterized by an increase in cAMP levels, we analyzed early responses to define the relationship between cAMP and ANP release. Before histamine, ANP concentration was 0.530 ± 0.073 μM and decreased by 50.69 ± 4.50% (mean of the last two periods) to 0.249 ± 0.036 μM after three cycles of histamine (Fig. 4). The effects of histamine on ANP release, cAMP production, and atrial dynamics were concentration-dependent (Fig. 4). For the time-matched control, changes in ANP secretion, ECF translocation, ANP concentration, cAMP production, and atrial dynamics were constant.

Fig. 7. Effects of 8-Br-cAMP (0.6 mM) on ANP secretion (Aa), ECF translocation (Ab), ANP concentration (Ac), atrial stroke volume (Ad), and pulse pressure (Ae) in perfused beating rabbit atria (1.3 Hz; n = 9). B: effects of KT5720 (KT; 6 μM) on 8-Br-cAMP induced decrease in ANP secretion, ECF translocation, ANP concentration, atrial stroke volume, and pulse pressure (Bb–Be, respectively) (n = 6). †P < 0.05, +++P < 0.001 vs. control. ***P < 0.001 vs. KT5720.
and stable (Fig. 2B). The responses were reproducible during the periods corresponding to the control and experimental observations (differences between the periods were not significant; n = 10).

Histamine decreases ANP release via H2 receptor. To define the receptor responsible for the effect of histamine on the regulation of ANP release, an antagonist of the histamine H2 receptor was applied. An inhibition of histamine H2 receptor with cimetidine completely blocked all effects of histamine on ANP release, cAMP production, and atrial dynamics (Figs. 5A and 6). Group for the experiment of histamine shown in Fig. 5A is different from that of Fig. 2. The effects of histamine were very similar in both groups. Cimetidine alone did not show significant changes in the above atrial parameters (Fig. 6). In contrast to the H2-receptor antagonist, the H1-receptor antagonist triprolidine did not block the histamine-induced decrease in ANP release and increase in atrial dynamics (Figs. 5B and 6). In the presence of triprolidine, histamine increased cAMP production significantly, but the response was slightly attenuated (Fig. 5B). At the peak difference of the second cycle of histamine in the presence of triprolidine, the differences over the mean of the two periods before histamine were significant between the presence and absence of triprolidine (in difference in percent changes, 80.74 ± 19.37% for triprolidine plus histamine, n = 9 vs. 187.02 ± 33.90% for histamine, n = 13; P < 0.05). Triprolidine alone did not significantly affect the cAMP concentration (0.072 ± 0.013 µM for triprolidine vs. 0.050 ± 0.007 µM for control period, n = 7; P > 0.05). Triprolidine alone decreased ANP release at the later phase of the treatment and increased atrial dynamics at the early phase slightly but significantly (Fig. 6).

8-Br-cAMP mimicks the effect of histamine on ANP release. To define the role of cAMP in the regulation of ANP release, a cell-permeable cAMP analog, 8-Br-cAMP, was infused. 8-Br-cAMP decreased ANP secretion and ANP release (Fig. 7, Aa and Ac). 8-Br-cAMP increased atrial stroke volume and pulse pressure (Fig. 7, Ad and Ae). 8-Br-cAMP decreased ANP release and increased atrial dynamics in a dose-dependent manner (Fig. 8, A and B). As shown in Fig. 7B, KT5720, a PKA selective inhibitor, blocked 8-Br-cAMP-induced decrease in ANP release and increase in atrial dynamics [ANP concentration (in µM): -4.28 ± 3.48, n = 4, vs. -39.72 ± 2.30, n = 9; P < 0.001].

Inhibition of L-type Ca2+ channel with nifedipine failed to change histamine-induced decrease in ANP release. Because the H2 receptor-activated increase in cAMP production is expected to increase Ca2+ influx via L-type Ca2+ channels (19), and increase in Ca2+ influx via L-type channel is an inhibitory regulator for ANP release (9, 21, 34, 39), the effect of Ca2+ channel blockade on histamine-induced decrease in ANP release was tested. Nifedipine, an inhibitor of L-type Ca2+ channel, increased ANP release with a concomitant decrease in atrial dynamics (Fig. 9, Aa, Ac, Af, Ag, Ba, Bc, Bf, and Bg). The effects of nifedipine were stable and maintained during the periods of experiments. Nifedipine decreased ECF translocation slightly but significantly, which was coincident with a decrease in atrial dynamics (Fig. 9, Ab and Bb). Nifedipine had no significant effect on cAMP efflux (Fig. 9, Bd and Be). In the presence of nifedipine, histamine decreased ANP release concomitantly with an increase in cAMP efflux (Fig. 9, Aa and Ac–Ac). Nifedipine failed to change histamine-induced decrease in ANP release (in difference in percent changes, -41.79 ± 4.34% for nifedipine plus histamine, n = 7 vs. -50.69 ± 4.50% for histamine, n = 10; P > 0.05). Nifedipine attenuated histamine-induced increase in atrial stroke volume and pulse pressure (Fig. 9, Af and Ag).

Protein kinase inhibitor staurosporine blocks histamine-induced decrease in ANP release. As shown in Fig. 10, staurosporine (0.1 µM) slightly but not significantly decreased atrial ANP release (Fig. 10, Aa, Ac, Ba, and Bc). Staurosporine significantly decreased atrial dynamics (Fig. 10, Af, Ag, Bf, and Bg). In the presence of staurosporine, histamine-induced decrease in ANP release was not observed (Figs. 10, Aa and Ac, and 11). Staurosporine (0.03 µM) attenuated histamine-induced decrease in ANP release (in difference in percent changes, 10.92 ± 3.05% for histamine + staurosporine vs. 0.00 ± 0.06% for staurosporine, n = 10; P < 0.05).

Fig. 8. Concentration-dependent effects of 8-Br-cAMP on the changes in ANP concentration (A) and atrial stroke volume (B). The responses were compared with differences of mean values of 2 fractions before (fraction numbers 11 and 12) and after 3 cycles (fraction numbers 29 and 30; for the atrial stroke volume 23 and 24) of 8-Br-cAMP or vehicle. Number of experiments: 8-Br-cAMP (0.3 mM), n = 7; 8-Br-cAMP (0.6 mM), n = 9; 8-Br-cAMP (1.0 mM), n = 5; control, n = 6. Data for 8-Br-cAMP (0.6 mM) were derived from Fig. 7A.
mine-induced decrease in ANP release (Fig. 11). Stau-
rosporine attenuated histamine-induced decrease in
ANP release in a concentration-dependent manner.
The change in ANP concentration by staurosporine (0.1
μM) plus histamine was not significantly different
from those by staurosporine alone. Staurosporine
blocked histamine-induced increase in atrial dynamics
(Fig. 10, Af and Ag). Histamine induced a slight but not
significant increase in ANP secretion (Fig. 10Aa),
which may be related with an increased ECF translo-
cation. In the presence of staurosporine, histamine
significantly increased cAMP production (Fig. 10, Ad
and Ae). Histamine-induced increase in cAMP concen-
tration was not different between the presence and
absence of staurosporine (232.94 ± 59.71% for stauro-
sporine, 0.1 μM, plus histamine, n = 7, vs. 220.93 ±
54.39% for histamine, n = 10; P > 0.05). Staurosporine
significantly shifted the relationship between cAMP
and ANP release (in changes in slopes; −0.035 ± 0.023
for staurosporine plus histamine, n = 6 vs. −0.133 ±
0.024 for histamine, n = 10; P < 0.05).

Because the histamine-induced decrease in ANP re-
lease was found to be a function of increase in cAMP
efflux and also staurosporine blocked the response,
involvement of PKA activity was de-

Fig. 9. Effects of nifedipine (Nife; 1.0 μM) on
Hist-induced decrease in ANP secretion (Aa),
ECF translocation (Ab), ANP concentration (Ac),
cAMP efflux (Ad), cAMP concentration (Ae),
atrial stroke volume (Af), and pulse pressure
(Ag) in perfused beating rabbit atria (1.3 Hz; n =
7). B: effects of Nife (1.0 μM) on the same param-
eters (Ba–Bg) were stable during the period cor-
responding to Hist infusion (n = 6). *P < 0.05,
**P < 0.01 vs. Cont period; *P < 0.05, **P <
0.01 vs. Nife (values of the 1 cycle before the
administration of Hist or vehicle).
response was significantly attenuated (Figs. 12, Aa and Ac, and 15). In the presence of KT5720, histamine increased cAMP efflux significantly, but the response was attenuated. Difference in percent changes in cAMP concentration induced by histamine was not significantly different between the presence and absence of KT5720 (194.66 ± 11005 57.08% for KT5720 plus histamine, n = 9 vs. 220.93 ± 11006 54.39% for histamine, n = 10; P > 0.05). However, KT5720 attenuated histamine-induced accentuation of cAMP concentration in the absolute amount of changes (0.040 ± 0.007 μM for KT5720 plus histamine, n = 9 vs. 0.089 ± 0.013 μM for histamine, n = 10; P < 0.01). KT5720 attenuated histamine-induced increase in atrial stroke volume and pulse pressure. A lower concentration of KT5720 (3 μM) failed to modulate histamine-induced decrease in ANP release (Fig. 13). Up to 10 μM of KT5720, the attenuation by KT5720 of the histamine-induced decrease in ANP release was not different from that by 6 μM of KT5720 (Fig. 13). RP-cAMP[S], another structurally different PKA inhibitor, did not significantly influence ANP release and atrial dynamics (Figs. 14B and 15). In the presence of RP-cAMP[S], histamine decreased ANP secretion and ANP concentration, but the response was attenuated (Figs. 14, Aa and Ac, and 15). RP-cAMP[S] attenuated histamine-induced accentuation of atrial dynamics (Figs. 14, Ad and Ae, and 15). In this experiment, the response of cAMP could not be determined because the specific antiserum for the radioimmunoassay cross-reacted with RP-cAMP[S].

**DISCUSSION**

The present study clearly shows that histamine H₂ but not histamine H₁-receptor activation with hista-
Histamine decreases atrial ANP release with concomitant increases in cAMP production and atrial dynamics.

**Histamine decreases ANP release via H2-receptor activation.** Histamine results in a decrease in ANP release as well as increases in cAMP production and atrial dynamics in a concentration-dependent manner. The histamine H2 but not H1-receptor antagonist blocked effects of histamine. Triprolidine, a H1-receptor antagonist did not block histamine-induced changes in ANP release, cAMP production and atrial dynamics. Previously, Hattori et al. (18) showed in rabbit atria that histamine-induced accentuation of atrial dynamics is exclusively mediated by H2 receptor. They also showed that H2-receptor activation with histamine increased cAMP production in rabbit atria. The present data are in agreement with the report. The present study suggests that increase in atrial cAMP production by H2-receptor activation is related to the
Histamine-induced decrease in ANP release. In the present study, it was shown that triprolidine alone slightly but significantly increased atrial dynamics and decreased ANP release. Triprolidine attenuated histamine-induced accentuation of cAMP production. The mechanism by which triprolidine elicits the effects is not clear at present.

**Mechanism by which H₂-receptor activation regulates ANP release.** The present study shows for the first time that an activation of G protein-coupled histamine H₂ receptor decreases atrial ANP release via cAMP-protein kinase signaling. Histamine-induced decrease in ANP release was a function of cAMP production. Because an accentuation of cAMP production results in an activation of L-type Ca²⁺ channels (14, 19, 31) and also an increase in Ca²⁺ influx via L-type channels inhibits ANP release in the beating atria and perfused heart (9, 21, 34, 39), it was expected to observe a role for increased Ca²⁺ influx in the cAMP inhibition of ANP release by histamine. However, histamine-induced decrease in ANP release was not modified by an L-type Ca²⁺ channel inhibitor, nifedipine. An increase in ANP secretion by an L-type Ca²⁺ channel inhibitor is consistent with the previous report (39). Nifedipine blocked histamine-induced accentuation of atrial dynamics. This is consistent with previous reports (14, 19, 31).

A protein kinase nonselective inhibitor, staurosporine, blocked histamine-induced decrease in ANP release in a concentration-dependent manner. In this condition, histamine increased atrial cAMP produc-

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**Fig. 13.** Concentration-dependent effect of KT on Hist-induced decrease in ANP release. For KT (10 µM) plus Hist group, 3 of 8 experiments were done with 20 µM KT, and the values obtained were not different from those of the atria treated with 10 µM KT. For the KT (6 µM) alone group, 2 of 7 experiments were done with 20 µM KT, and the values obtained were not different from those of the atria treated with 6 µM KT.

**Fig. 14.** Effects of RP-cAMP[S] (RP; 125 µM) on Hist-induced decrease in ANP secretion (Ad), atrial stroke volume (Ac), and pulse pressure (Af) in perfused beating rabbit atria (1.3 Hz; n = 3). B: effects of RP (125 µM) on the same parameters (Ba–Be) were stable during the periods corresponding to the Hist infusion except atrial dynamics, which are slightly but significantly decreased at the end of experiments (n = 3).

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 Histamine \( H_2 \) receptor-mediated decrease in ANP release

Fig. 15. Comparison of effects of protein kinase A inhibitors on ANP concentration and atrial dynamics. Values are the difference in percent changes over the mean value of 2 periods before Hist. Number of experiments: Hist alone (10 \( \mu \)M, \( n = 10 \)); KT plus Hist (6 \( \mu \)M, \( n = 9 \)); RP plus Hist (125 \( \mu \)M, \( n = 4 \)); KT plus vehicle (6 \( \mu \)M, \( n = 5 \)); RP plus vehicle (125 \( \mu \)M, \( n = 3 \)); time-matched control (\( n = 10 \)).

\( \Delta \% \) changes in stroke volume

Hist KT + Hist Rp + Hist KT + Vehicle Rp + Vehicle Control

\( \Delta \% \) changes in ANP concentration

ANP Hist KT + Hist Rp + Hist KT + Vehicle Rp + Vehicle Control

H2 receptor-cAMP signaling via PKA-dependent and PKA-dependent and -independent pathways. Previously, it was shown that cAMP-coupled histamine \( H_2 \) receptor of the cardiac atrium inhibits secretory function via cAMP-protein kinase signaling and accentuates atrial dynamics via cAMP-Ca\(^{2+} \) pathway. This notion is in relation with the previous report that showed a Ca\(^{2+} \) channel-independent and cAMP-protein kinase-dependent signaling for forskolin-induced inhibition of ANP release (7).

Previously, it was shown that cAMP-elevating agents increased ANP secretion in cultured cardiac myocytes (5), isolated atria (1, 36), or perfused hearts (33). The present data contrast with these reports. The discrepancy between the present study and previous reports may be related to the methodology. The most important stimulus for the regulation of ANP secretion is atrial stretch (11, 24). We have shown that ANP secretion is regulated by a two-step sequential mechanism (2, 3). First, ANP is released from atrial myocytes into the surrounding extracellular space. Second, convective transendocardial translocation of the ANP-containing ECF into the bloodstream is induced by atrial contraction. The convective translocation of the ECF as the final step of ANP secretion was shown to be dependent on the atrial workload. Therefore, an increase in atrial workload would be expected to accentuate ANP secretion by increasing the translocation of ECF. Recently, it was also shown that atrial workload inversely determined the size of extracellular space (4). The latter directly determined the translocation of the ECF in terms of atrial workload. Therefore, it is suggested that the size of extracellular space and also atrial workload are important in the regulation of the translocation of ECF and released ANP. This may also be implicated in the cultured cardiocytes. This notion may be relevant to the discrepancy between the present study and previous reports.

Recently, it was shown that acute stress induces cardiac mast cell activation and histamine release (20). Also, it has been known that cardiac mast cell-derived histamine can constrict coronary artery (15). Therefore, forskolin-induced accentuation of cAMP production was not modulated by protein kinase nonselective and PKA selective inhibitors staurosporine and KT5720, respectively (7). Therefore, it is suggested that the site of modulation by protein kinase inhibitor of the histamine-induced increase of cAMP concentration is upstream of the adenylyl cyclase. However, histamine \( H_2 \)-receptor desensitization may not be involved in this process, because desensitization of G protein-coupled receptor is known to be related with phosphorylation of the receptor by PKA activation (16, 27) and also because PKA is reported not to be involved in the \( H_2 \)-receptor desensitization (10, 26, 37).

Taken together, the present study suggests that histamine decreases atrial ANP release via L-type Ca\(^{2+} \) channel-independent and PKA-dependent and -independent signaling. This is consistent with the hypothesis that an increase of Ca\(^{2+} \) influx by \( H_2 \)-receptor activation by histamine is mainly involved in the control of atrial dynamics but not in the regulation of ANP release. Therefore, these data suggest that an activation of G protein-coupled histamine \( H_2 \) receptor of the cardiac atrium inhibits secretory function via cAMP-protein kinase signaling and accentuates atrial dynamics via cAMP-Ca\(^{2+} \) channel pathway. This notion is in relation with the previous report that showed a Ca\(^{2+} \) channel-independent and cAMP-protein kinase-dependent signaling for forskolin-induced inhibition of ANP release (7).
fore, the present study showing histamine-induced decrease in ANP release implicates the pathophysiology of cardiac diseases accompanying cardiac mast cell activation (22). In such a pathological condition as that mentioned above, which accompanies mast cell activation and increase in histamine release, histamine-induced decrease in ANP release may aggravate the coronary dysfunction. Because ANP is known to vasodilate the coronary vasculature, we propose that histamine-induced decrease in ANP release may, at least in part, lead to impaired coronary vasodilation.

In summary, the present study shows that an activation of histamine \( \text{H}_2 \) receptor adenyl cyclase-coupled receptor by histamine decreases atrial ANP release, in which an elevation of cAMP production regulates ANP release via cAMP-protein kinase signaling.

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DISCLOSURES

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