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Role of Histamine in the Regulation of Coronary Circulation

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Summary

The aim of this study was to evaluate the role of endogenous histamine in the regulation of reactive hyperaemia (RH) and coronary autoregulation in isolated rat hearts. The basal release of cardiac histamine (perfusion pressure 60 cm H_2O) amounted to 100–200 pmol/min/g wt. During the first 15 s following 30 s of coronary occlusion, the release of histamine increased about three times and returned to basal levels after approximately 90 s, paralleling the changes of coronary flow (CF). Blockade of H_1-receptors increased basal CF by 23±2 %, significantly reduced blood flow debt and prolonged the duration of RH. Blockade of H_2- and H_3-receptors produced a significant decline of CF, decreased RH flow and diminished RH by 40±3 %. Blockade of all three classes of histamine receptors indicated that endogenous histamine exerts predominantly vasodilatory effects (mediated by H_2- and H_3-receptors) on coronary circulation. Histamine-induced vasodilation appears to be NO-dependent. Changes of coronary perfusion pressure from 20 to 120 cm H_2O were accompanied by an almost linear decrease of histamine release from about 200 to 40–50 pmol/min/g wt. Blockade of histamine receptors decreased, while L-NAME significantly widened the autoregulatory range of the isolated rat heart, reduced CF and release of NO, but reversed the pattern of histamine release leaving the autoregulatory range unaltered, which indicate that endogenous histamine does not play a role in the regulation of coronary autoregulation.

Key words

Histamine – Coronary reactive hyperaemia – Coronary autoregulation – Histamine receptors – Nitric oxide

Introduction

The cardiovascular effects of histamine have been recognized soon after its discovery (Dale and Ladlow 1910). In most animal species, the primary and direct response of the heart to histamine is characterized by an increase in rate and contractility, a decrease in atrioventricular nodal conduction velocity, enhanced automaticity and an increase in coronary flow rate. The available data suggest that many of the cardiac effects of histamine are multifactorial. Thus, a particular response is the result of multiple components mediated by three types of histamine receptors, as well as the result of various interactions with other autacoids (Hill 1990, Levi *et al.* 1991, Kostić and Petronijević 1995).

Metabolic regulation plays the dominant role in the regulation of coronary circulation. The list of metabolites that have been supposed to regulate coronary flow (CF) and hence affect coronary reactive hyperaemia is long. Among them, adenosine, adenine nucleotides, carbon dioxide, prostaglandins and nitric oxide were considered as the most important (Marcus 1983, Borst and Schrader 1991, Kostić and Schrader 1992). However, involvement of these metabolites in coronary autoregulation is still controversial (Dole *et al.* 1985). Endogenous histamine, as one of them, has been indicated almost 20 years ago (Olsson 1975) as a potential mediator of reactive hyperaemia. This assumption has been confirmed recently in the isolated guinea-pig heart (Rosić *et al.* 1993). However, the role of endogenous histamine in coronary autoregulation remains unknown.

This study was undertaken in order to explore the contribution of cardiac histamine to coronary reactive hyperaemia (RH) and coronary autoregulation. Furthermore, the interactions of cardiac histamine with other cardiac autacoids, mainly nitric oxide and adenosine, were studied. The results presented here confirm the role of histamine in the

regulation of coronary vascular tone under basal and reactive hyperaemia conditions, but not during autoregulation.

Materials and Methods

Hearts, isolated from Wistar albino rats of 200–300 g body mass, were perfused at constant pressure according to the Langendorff technique. The perfusion medium, equilibrated with 95 % O_2+5 % CO_2 at 37 °C, contained (mmol/l) NaCl 118, KCl 4.7, $NaHCO_3$ 25, $CaCl_2$ 2.5, $MgSO_4$ 1.7, KH_2PO_4 1.2, glucose 11, pyruvate 2. All hearts were electrically paced (4 V, 320 bpm). Constant left ventricular draining through the dissected mitral valve was provided.

After the isolated heart perfusion had been set up, 30 min were allowed for stabilization of the preparation. Reactive hyperaemia was induced by occlusion of coronary inflow for 30 s. After each occlusion, 10–15 min were allowed to permit the heart to attain a new haemodynamic steady state. Samples of coronary effluent perfusate were collected for 30 s before and during the first 90 s after coronary occlusion. Collection time for every sample was 15 s. The same protocol was followed with hearts perfused with 1 nmol/l pyrilamine (H_1-histamine receptor antagonist), 300 nmol/l ranitidine (H_2-antagonist) plus 10 nmol/l thioperamide (H_3-antagonist) or all three compounds in the above concentrations. The final concentrations of histamine antagonists were chosen according to their K_d values (Hill 1990). All antagonists were infused directly into the perfusion cannula to which the heart was attached. The hearts were equilibrated for 5 min with each antagonist before the first effluent perfusate samples were collected.

In the autoregulation experiments after initial equilibration, performed at a pressure of 60 cm H_2O, the coronary perfusion pressure was lowered to 50, 40, 30 and 20 cm H_2O, then increased gradually in the reverse order to 60 cm H_2O and further to 70, 80, 90, 100, 110 or 120 cm H_2O. When flow was estimated as stable at each value of perfusion pressure, samples of the coronary effluent were collected. At the end of this series of pressure changes (basic protocol), the hearts were perfused with L-NAME (30 μmol/l), as an inhibitor of NO synthesis (Emery 1995), either with all three histamine receptor antagonists together, and the perfusion pressure was changed as in the basic protocol.

Histamine determination was based on the fluorimetric procedure of Shore *et al.* (1959) as modified by Bergendorff and Uvnäs (1972). Nitric oxide was assessed as nitrite and quantified by the spectrophotometric method using the Griess reagent (Green *et al.* 1982).

Statistical significance of differences was estimated using Student's paired t-test.

Table 1
Changes of coronary flow, histamine and nitrite release before and during reactive hyperemia after 30 s of coronary occlusion in the isolated rat heart

Time s	Coronary flow ml/min/g	Histamine release pmol/min/g	Nitrite release nmol/min/g
Before occlusion			
30–16	7.80±0.56	122.67±24.22	3.76±1.55
15–0	7.74±0.54	126.68±22.12	3.74±1.53
After 30 s occlusion			
0–15	10.80±0.75[c]	364.67±48.34[c]	10.66±2.95[b]
16–30	9.58±0.49[c]	267.67±17.46[c]	3.54±0.47
31–45	8.91±0.72[a]	244.33±88.43[a]	1.78±0.59[a]
46–60	8.47±0.84	210.33±63.97[a]	2.51±0.39
61–75	8.03±0.71	171.33±31.25[a]	2.75±0.58
76–90	7.73±0.57	110.67±13.93	3.75±1.54

Values are means ± S.E.M. of 11 experiments. Statistical significance in comparison to preocclusion values: a) $p<0.05$; b) $p<0.01$; c) $p<0.001$.

Results

In the first series of our experiments on isolated rat hearts perfused at a constant pressure of 60 cm H_2O, the mean CF was 7.8±0.56 ml/min/g wt (n=11). Coronary occlusion of 30 s induced RH which lasted 67.5±2.6 s on the average. This is close to the values reported for guinea-pig hearts (Kostic and Schrader 1992). The basal release of histamine amounted to 122.67±24.22 pmol/min/g wt. (Table 1) which is only 1/3 of the value obtained in guinea-pig hearts (Rosic *et al.* 1993). During the first 15 s after 30 s of coronary occlusion, the flow increased by about 40 %, while the release of histamine and nitrite increased about three times (Table 1). Thereafter, the release of histamine gradually declined reaching preocclusive values after about 90 s. After the initial 3-fold increase, the release of nitrite declined rapidly and reached preocclusive values after 30 s of reperfusion (Table 1). Interestingly, the release of nitrite continued to decline, but again slowly reached preocclusive values after 90 s.

In order to estimate the role of cardiac histamine in the regulation of RH, selective antagonists of histamine receptors were applied. These experiments showed a significant increase of basal CF by 23±2 % in the presence of pyrilamine (1 nmol/l), accompanied with reduced flow repayment and prolonged duration of RH (78.7±3.3 s vs 58.7±1.2 s). Ranitidine (300 nmol/l) in combination with thioperamide (10 nmol/l), i.e. simultaneous blockade of H_2- and H_3-receptors, produced a significant decline of CF under basal conditions (6.03±0.93 vs 7.43±0.58 ml/min/g), a decrease of flow during RH as well as a significant reduction of RH by 40±3 %. As was to be expected, the combination of all three histamine receptor antagonists lowered basal CF by 12±2 %, reduced RH flow and shortened the duration of RH (47.5±2.5 vs 67.5±4.3 s). These changes of CF flow and RH were accompanied by the following changes of nitrite release: H_1-blockade increased basal release of nitrite by 86.8±5.1 %, H_2+H_3-blockade decreased it by 74.2±10.1 %, while $H_1+H_2+H_3$-blockade decreased it by 48.6±9.1 %.

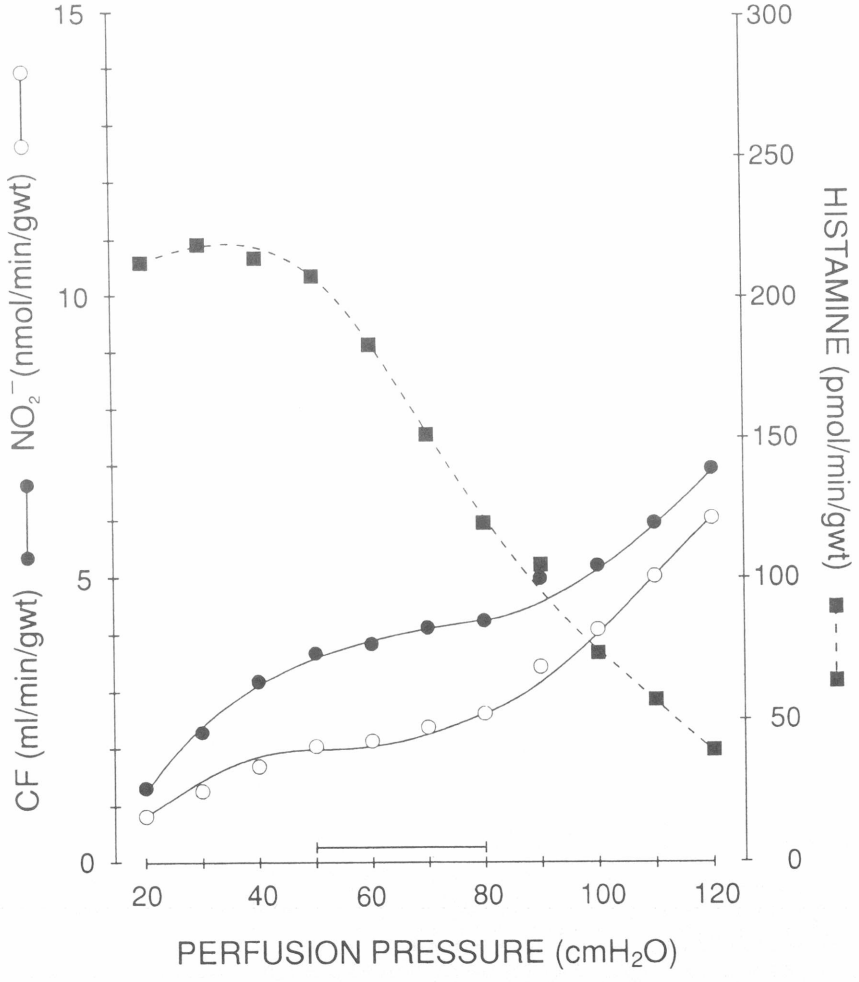

Fig. 1
Coronary autoregulation in the rat heart: Changes of coronary flow (CF) (full circles), nitrite (open circles) and histamine (full squares) release. Horizontal bar denotes autoregulatory range (50–80 cm H_2O). Each point represents mean values of 11 paired experiments.

According to the results presented in Figure 1, isolated rat hearts exhibited the ability to autoregulate CF in the perfusion pressure range of 50–80 cm H_2O. In this series of experiments, the release of histamine at 60 cm H_2O was higher and amounted to 189±74 pmol/min/g wt, which may be explained by the age-dependent release of histamine (Moritoki *et al.* 1988). The decrease of perfusion pressure was accompanied by an increase of histamine release up to 216±75 pmol/min/g wt (at 40 cm H_2O), while raising of perfusion pressure led to an almost linear drop of histamine release to 40–50 pmol/min/g wt (above 100 cm H_2O). However, throughout the whole range of perfusion pressure changes, the release of nitrite strictly paralleled the changes of CF with a marked tendency to increase above the autoregulatory range (Fig. 1).

Perfusion with L-NAME, but not D-NAME, significantly decreased CF over the entire range of perfusion pressure changes and widened coronary autoregulation to a pressure range from 40 to 100 cm H_2O (Fig. 2A). Blockade of all three classes of histamine receptors diminished the capacity of coronary autoregulation (Fig. 2B). Since the obtained changes of histamine release resemble those of adenosine release (Schrader 1977), perfusion with theophylline (30 μmol/l) was performed. In the presence of theophylline, CF was reduced by 15–20 % and the pressure flow curve was shifted downwards, but without significant changes of coronary autoregulation (Fig. 2A). Perfusion with theophylline reduced nitrite release by about 45 % at lower pressures (30–70 cm H_2O) and by 25 % at 120 cm H_2O. However, the release of histamine in the presence of theophylline was almost completely blocked at 20 cm H_2O, while it increased by about 80 % at 120 cm H_2O, exhibiting a completely inverse pattern as compared to control hearts (Fig. 2C). Interestingly, the release of histamine between 60 and 100 cm H_2O is not significantly altered by theophylline (Fig. 2C).

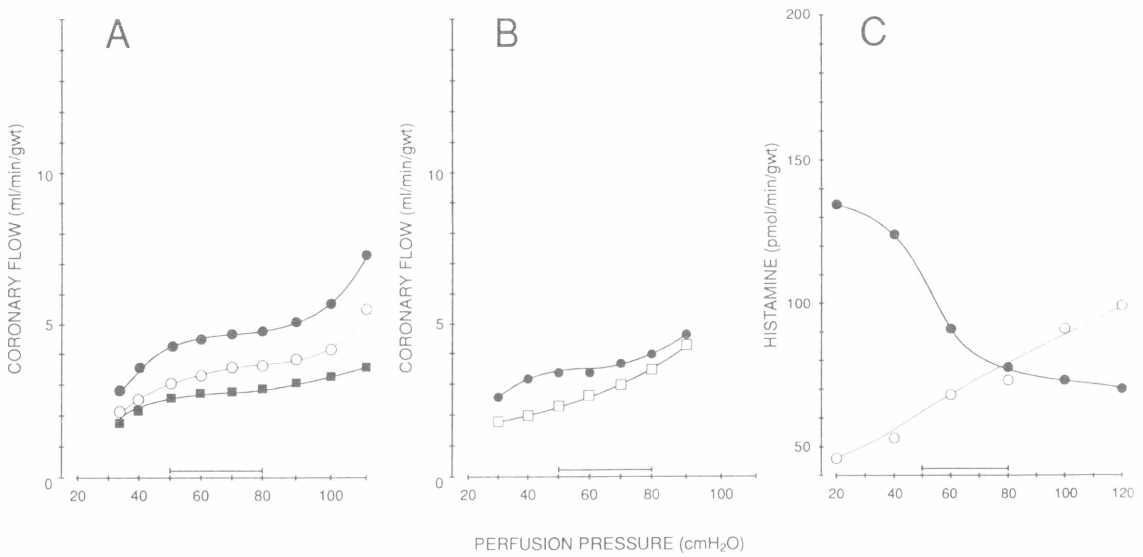

Fig. 2

Coronary autoregulation in the rat heart: A) Pressure flow curves in the control hearts (full circles) and hearts perfused with 30 μmol/l theophylline (open circles) and 30 μmol/l L-NAME (full squares), respectively. B) Pressure-flow curves in control hearts (full circles) and hearts perfused with antagonists of all three classes of histamine receptors (open squares). C) Changes of histamine release in control (full circles) and hearts perfused with 30 μmol/l theophylline (open circles). Values are means of 5 (A, B) and 7 (C) paired experiments, respectively.

Discussion

This work has demonstrated the regulation of RH by histamine in the isolated rat heart. Several lines of evidence presented in this study support this conclusion.

1) During RH, there is a 66–188 % increase of histamine as compared to basal conditions (Table 1).
2) The maximal, almost a 3-fold, increase of histamine release coincides with the peak of RH flow,

i.e. with maximal vasodilation and the nadir of coronary resistance.

3) The kinetics of histamine release almost parallel the changes of CF during RH (Table 1).

4a) Ranitidine and thioperamide, H_2- and H_3-antagonists, attenuate RH, as demonstrated by reduced flow as well as the duration of RH (see Results).

4b) Pyrilamine, H_1-receptor antagonist reduces RH flow repayment and prolongs RH in isolated rat hearts.

4c) Blockade of all three classes of histamine receptors reduce flow during RH and shorten the duration of RH. Therefore, on the basis of these findings it could be concluded that histamine regulation of RH is a receptor-mediated event. In this respect isolated rat hearts are similar to guinea-pig hearts, which may be of importance since there are many species dependent on the cardiovascular effects of histamine (Levi *et al.* 1991).

The quantities of histamine released by unstressed rat hearts perfused at a pressure of 60 cm H_2O vary between 100 and 200 pmol/min/g wt, depending very much on the animal's age (Moritoki *et al.* 1988). Values reported for isolated guinea-pig hearts are up to three times higher (Rosić *et al.* 1993, Kostić and Petronijević 1995), also indicating a species-dependence (Levi *et al.* 1991). According to the results of our study, the obtained histamine concentrations of 10–20 nmol/l appear sufficient to control basal coronary tone. This is supported by two of our findings. Blockade of H_1-receptors significantly increases basal CF by about 25 %, while H_2- and H_3-antagonists significantly decrease basal CF. Obviously, the increase of CF in the presence of H_1-antagonist occurs due to the unopposed stimulation of H_2- and H_3-receptors by endogenous histamine. On the contrary, the decrease of CF produced by ranitidine plus thioperamide, appears as the consequence of H_1-receptor mediated vasoconstriction. These findings are in accordance with the earlier assumption that stimulation of H_1-receptors in the coronary bed produces vasoconstriction (Black *et al.* 1986), while that of H_2- and H_3-receptors induces coronary vasodilation (Reinhardt and Ritter 1979, Andjelković *et al.* 1991). The obtained changes of basal CF, as well as of RH, when all three classes of histamine receptors are blocked, indicate a predominant coronary vasodilatory effect of histamine under physiological conditions. This means that H_2- and H_3-mediated vasodilation prevails over H_1-mediated coronary vasoconstriction. This conclusion is in accordance with the observation that a histamine bolus provokes dose-dependent vasodilation in the isolated guinea-pig heart (Levi *et al.* 1990).

Histamine-induced coronary dilation in the isolated guinea-pig heart is nitric oxide-dependent (Levi *et al.* 1990). Measurements of nitrite released during perfusion of guinea-pig hearts with histamine antagonists was significantly increased by pyrilamine (due to unopposed binding of histamine to H_2- and H_3-receptors) and greatly reduced by cimetidine and thioperamide (Kostić and Petronijević 1995). The results presented in this work indicate that H_2- and H_3-mediated coronary vasodilation is NO-dependent. Furthermore, the blockade of all three classes of histamine receptors halves nitrite release, which is accompanied by 12 % reduction of basal CF as well as attenuation of RH. Therefore, one may assume that increased histamine release contributes to the increased release of NO during RH. Furthermore, NO potentiates the release of myocardial histamine, indicating a positive feedback between these two autacoids in the regulation of coronary circulation during basal and RH conditions (Kostić and Petronijević 1994, 1995).

It is well known that autoregulation reflects the interaction of myogenic tone with the opposing effect(s) of one or more endogenous vasodilators (Dole *et al.* 1985). One such metabolite, adenosine which plays an important role in mediation of RH, appears to have no role in setting coronary tone within the autoregulatory range (Hanley *et al.* 1986). However, it is important below the autoregulatory range when the oxygen supply could not meet the oxygen demands (Bardenheuer and Schrader 1983, Johnson 1986). Changes of histamine release during a change of perfusion pressure from 20 to 120 cm H_2O (Fig. 1) resemble changes of adenosine release (Schrader *et al.* 1977), as well as of hypoxanthine and xanthine – degradative products of adenosine metabolism (unpublished results). However, the blockade of histamine receptors diminishes the ability of coronary circulation to autoregulate by exerting more prominent reduction of CF below the autoregulatory range (Fig. 2B).

Theophylline, which blocks coronary vascular action of adenosine and ATP equally well (Borst and Schrader 1991), does not influence coronary autoregulation in rat hearts (Fig. 2A) as expected, since adenosine has no role in coronary autoregulation. However, theophylline reduced NO release (Kostić and Schrader 1992), which should be accompanied by increased coronary autoregulation as shown here (Fig. 1A), as well as by others (Ueeda *et al.* 1992, Smith and Canty 1993). In our experiments, this was not the case, probably due to the pressure-dependent theophylline-induced reduction of NO release which is, according to our unpublished results, twice as high at pressure of 30–70 cm H_2O than at 120 cm H_2O. Furthermore, the blockade of adenosine action, which according to earlier results may stimulate the release or/and the effects of histamine (Wiedmeier and Spell 1977, Hill and Kendall 1987), increases histamine release at pressure values above 80 cm H_2O (Fig. 2C). These data taken together, question the role of histamine in

coronary autoregulation in rat hearts, although our earlier data indicate that it may have a slight modulatory role on coronary autoregulation in guinea-pig hearts (Kostić and Petronijević 1995).

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