Histamine Receptors in the Coronary Circulation of the Dog

Effects of Mepyramine and Metiamide on Responses to Histamine Infusions

ROBERT W. GILES, GEOFFREY HEISE, AND DAVID E.L. WILCKEN

SUMMARY The effect of histamine on coronary blood flow (CBF) was studied in anaesthetized greyhounds. CBF and systemic blood pressure were measured using electromagnetic flow transducers and catheters in the aorta during infusions of drugs into the left circumflex coronary artery. Histamine infusions (5, 10, and 20 µg/min) produced dose-related increases in CBF without changing heart rate or blood pressure. Metiamide (100 µg/min) given simultaneously produced a parallel displacement of the histamine dose-response curve to the right (P < 0.05) with a dose ratio of 2. Mepyramine (100 µg/min) produced a larger parallel displacement of the dose-response curve (dose ratio = 4). Together, metiamide and mepyramine greatly reduced the histamine response (dose ratio = 16), showing that the metiamide blockade is augmented in the presence of mepyramine. Similarly, mepyramine blockade is augmented in the presence of metiamide. The increase in CBF produced by histamine infusion (20 µg/min) was similar to the peak flow response of reactive hyperemia following 8-second occlusions. However, mepyramine and metiamide together had no effect on the peak flow response, duration, or total repayment of flow debt in reactive hyperemia. These results show that histamine-induced coronary vasodilatation is mediated by both H₁ and H₂ receptors. However, the vasodilation of reactive hyperemia after brief coronary artery occlusions does not appear to involve histamine.

HISTAMINE acts through at least two specific receptors.¹ ² The development of H₁ receptor antagonists³ and more recently of specific H₂ antagonists⁴ has made it possible to identify the presence in tissues of these two receptors. Histamine is taken up actively by the heart⁴ and its release may be quantified.⁵ ⁶ But the role of histamine in the regulation of the coronary circulation and the nature of coronary histamine receptors are unknown.

In the present investigation we have studied the action of histamine in the coronary circulation, using low dose close coronary arterial infusions of histamine and specific H₁ and H₂ receptor antagonists. By giving drugs in this way we avoided systemic effects. The results show that there are H₁ and H₂ receptors in the coronary vasculature with vasodilator effects similar in magnitude to that found during reactive hyperemia following brief coronary artery occlusions. We extended the study to examine the effects on myocardial reactive hyperemia of blocking histamine responses with both H₁ and H₂ receptor antagonists.

Methods

EXPERIMENTAL MODEL

Experiments were performed on anaesthetised healthy young greyhounds weighing 19-30 kg. Anaesthesia was induced with thiopentone (20 mg/kg, iv) and maintained by ventilation with a Harvard respirator using a 2:1 nitrous oxide-oxygen mixture and sodium pentobarbital. Sodium pentobarbital (20 mg/kg, iv) was given as an initial bolus and further doses were administered as necessary to suppress respiration. The nitrous oxide-oxygen gas mixture was passed through a humidifier to maintain body temperature at approximately 37.0°C. The respiratory rate and gas mixture were adjusted as necessary during the experiment to maintain blood gases and pH at physiological values.

Through a left thoracotomy, the pericardium was incised and the heart suspended in a pericardial cradle. The circumflex branch of the left coronary artery was dissected free for approximately 2 cm where there were no major branches. An appropriately sized [inside diameter (i.d.). 3-4 mm] IVM electromagnetic flow transducer (model FT24S) was placed around the exposed artery. A pneumatic occluder with an inflatable cuff was placed distal to the flow transducer. A polyvinyl catheter [i.d.. 0.4 mm; outside diameter (o.d.) 0.8 mm] was inserted through the circumflex coronary artery wall by a modification of the Herd and Barger technique⁶ to allow close arterial infusions of drugs. The catheter was passed in and out through the arterial wall and the catheter end was drawn through for approximately 30 cm. Both ends of the catheter were cut to equal length and infusions were passed through a small side hole in the intraluminal portion of the catheter. This allowed simultaneous infusions of two drugs. A polyvinyl catheter (1 mm. i.d.; 2 mm. o.d.) was passed into the abdominal aorta via the femoral artery for arterial blood pressure measurements. Coronary blood flow (CBF) was measured with an EMI electromagnetic flowmeter (SFMB-1 type 28). The signal to noise voltage ratio was 70:1. baseline drift less than 2%. and calibration accuracy.

Footnotes:

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Percent repayment of flow debt

Response. Flow debt, reactive hyperemic flow, and percent
compared only if resting CBF.

Reactive hyperemic flow

calculated as described by Coffman and Gregg according to the following formula:

\[
\text{Percent repayment of flow debt} = \frac{\text{reactive hyperemic flow}}{\text{flow debt}}
\]

In analysis of results, Student’s t-test was used to determine significance of differences between paired observations in each group of experiments. Results are expressed as means ± SE.

Results

In the 16 dogs studied, CBF was 81 ± 6 ml/min (range, 35-126 ml/min). Blood pressure was 130 ± 5 mm Hg (range, 95-152 mm Hg), and heart rate 183 ± 4 beats/min (range, 150-198 beats/min).

HISTAMINE

The doses of histamine used in this study were those found in preliminary studies to produce a dose-related response in the coronary circulation without producing systemic effects. Figure 1 is a representative experiment showing CBF responses to 1-minute intracoronary infusions of 5, 10, and 20 μg/min of histamine. CBF increase began about 20 seconds after the infusion commenced and reached a peak value at about 30 seconds. By allowing for the expected catheter transit time of 16 seconds, the CBF response to histamine occurred about 4 seconds after histamine had actually reached the coronary artery. In response to a histamine infusion of 5 μg/min, CBF rose from a resting value of 76 ml/min to a peak of 172 ml/min, an increase of 126%. The CBF returned rapidly to resting values on cessation of the histamine infusion. In response to a histamine infusion of 10 μg/min, the CBF rose from 72 ml/min to a peak of 213 ml/min, an increase of 196%. When histamine was infused at 20 μg/min, the peak increase was 253%. During each infusion, heart rate and blood pressure did not change. Following each infusion, CBF returned to resting values and a 5-minute interval was allowed before any further infusion was performed. An infusion of saline (0.9%) did not alter CBF, heart rate, or blood pressure.

To assess reproducibility of histamine responses, in three separate dogs infusions of histamine only at 5, 10, and 20 μg/min were given for the same number and duration of infusions used in histamine-blocking experiments. There was very little variation in five consecutive dose-response curves in each dog, with no evidence of tachyphylaxis to histamine. Histamine dose-response curves were performed twice in each preparation prior to the infusion of histamine blockers; there was no variation in responses in each dog.

MEPARYMINE

In preliminary experiments, mepyramine (100 μg/min) or metiamide (100 μg/min) given separately by intracoro-
nary infusion for 3 minutes did not affect heart rate, blood pressure, or CBF. When mepyramine and metiamide were given together for 3 minutes there was a small but insignificant reduction in CBF [4.5 ± 1% (mean ± se) in the 12 dogs studied]; but there was no change in heart rate or blood pressure. When mepyramine or metiamide were given at the dose of 1,000 µg/min for 3 minutes in separate dogs there was no significant increase in the blockade of the CBF response to histamine (20 µg/min) given by intracoronary infusion. With these large doses the preparations tended to deteriorate rapidly, therefore the lower doses of 100 µg/min of both mepyramine and metiamide were used.

HISTAMINE AND METIAMIDE

Figure 2 is a representative experiment showing the response to histamine (20 µg/min) given before and after a 3-minute infusion of metiamide. The metiamide infusion was commenced 2 minutes before histamine was given and continued during the 1-minute histamine infusion. Histamine increased CBF from 92 ml/min to a peak of 253 ml/min, an increase of 175%. Histamine (20 µg/min) in the presence of metiamide increased the flow by 103%. Finally, histamine (20 µg/min) in the presence of both metiamide and mepyramine increased flow by only 9%.

The group data showing the effects of histamine blockade in six dogs are summarized in Figure 3. CBF was 73 ± 12 ml/min (range, 35-104 ml/min) in these dogs. After metiamide there was a significant parallel displacement of the dose-response curve to the right (P < 0.05, paired observations) with a dose ratio of 2. Also seen in Figure 3 is the effect of combined blockade by metiamide and mepyramine after metiamide alone. The dose-response curve was shifted further to the right with a dose ratio of 16. At the highest dose of histamine (320 µg/min) used after simultaneous mepyramine and metiamide infusions, there were systemic effects. However, the small decrease in blood pressure and increase in heart rate occurred after the infusion of histamine had ceased, and recovered to normal values within 1 minute.

HISTAMINE AND MEPYRAMINE

Mepyramine (100 µg/min for 3 minutes) reduced the CBF response to histamine. A representative experiment is shown in Figure 4. Histamine (20 µg/min) increased CBF from 66 ml/min to a maximum flow of 255 ml/min. a

286% increase. When mepyramine (100 µg/min) was given 2 minutes before histamine and continued during the histamine infusion, histamine increased CBF by 63%. Finally, in the presence of both mepyramine and metiamide, the histamine response was almost abolished. There was only a 4% increase in flow.

The group data in six dogs are summarized in Figure 5. CBF was 81 ± 11 ml/min (range, 67-126 ml/min) in these dogs. There was a parallel significant (P < 0.01, paired observations) shift to the right of the histamine dose-response curve (dose ratio = 4). In the presence of combined mepyramine and metiamide blockade there was a further parallel shift to the right of the dose-response curve (dose ratio = 16). These combined histamine blockade responses are similar to those seen in the six dogs in which metiamide blockade was given initially (Fig. 3).

CONTRACTILITY CHANGES IN RESPONSE TO HISTAMINE

In two dogs left ventricular contractility during infusions was assessed using peak rate of change of left ventricular pressure (dP/dt). In the resting state, peak dP/dt measured for 10 consecutive beats was 1,749 ± 23 mm Hg/sec; during the histamine (20 µg/min) dP/dt was unchanged at 1,743 ± 21 mm Hg/sec. Likewise there were no contractility changes in response to histamine (40 µg/min) in the presence of mepyramine (100 µg/min) or to histamine.
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(160 μg/min) in the presence of both mepyramine (100 μg/min) and metiamide (100 μg/min). There was no change in heart rate or blood pressure in any of these experiments.

**REACTIVE HYPEREMIA WITH MEPYRAMINE AND METIAMIDE**

Histamine (20 μg/min) increased CBF to an extent similar to the increase caused by reactive hyperemia after an 8-second coronary artery occlusion; the peak flow increases were 150 ± 18% and 175 ± 27%, respectively. Reactive hyperemia following an 8-second occlusion was assessed before and after combined mepyramine and metiamide infusions. In five experiments in four dogs there was no significant alteration in the total repayment or the duration of hyperemia following the occlusion. The peak flow responses were not altered after infusions of mepyramine and metiamide infusions. In five experiments in four dogs there was no significant alteration in the total repayment or the duration of hyperemia following the occlusion. The peak flow responses were not altered after infusions of mepyramine and metiamide given simultaneously. The combined mepyramine and metiamide infusion was commenced 2 minutes before the 8-second occlusion, as was done with the histamine infusions, and continued until flow had returned to control values. The group data are shown in Table 1.

**Discussion**

The present study shows that histamine is a potent coronary vasodilator. Peak flow responses to close coronary arterial infusions of histamine without systemic effects are similar to those seen with intracoronary adenosine infusions. The maximum increase in CBF with histamine was similar to the increase with reactive hyperemia following brief coronary occlusions. The latter probably represents maximal coronary vasodilation. By using a coronary arterial catheter, we were able to give low-dose infusions which reached the vascular receptors without producing systemic effects. In the two dogs in which contractility changes were assessed, there was no alteration in contractility to the dose of histamine used by itself or in combination with the histamine antagonists. Thus, CBF responses were due only to the direct action of the infused drugs and not to metabolic changes secondary to contractility changes.

Powell and Brody recently described positive chronotropic and negative inotropic cardiac responses to intravenous histamine. These were not seen in our study with histamine and its antagonists given selectively into the circumflex branch of the left coronary artery. Thus we were able to avoid systemic effects which could produce reflex changes in the heart rate or contractility. Also, by selecting a site on the coronary artery distal to branches supplying the atria, we were able to avoid direct effects on pacemaking and conducting tissue in the heart. Although histamine was infused only into the circumflex artery, this supplies most of the free wall of the left ventricle so that contractility changes would have been recorded had there been significant inotropic effects.

The mean CBF in the present study (79 ± 11 ml/min) would have resulted in histamine concentrations of approximately 0.05 μg/ml, 0.15 μg/ml, and 0.25 μg/ml, respectively, with infusions of 5, 10, and 20 μg/min. Anrep and associates using dog heart-lung preparations in which histamine was injected at a concentration of 0.1 μg/ml of blood, found CBF increases similar to those reported here.

When histamine antagonists were given singly or in combination, dose-response curves were shifted to the right in a parallel manner, indicating competitive inhibition. Given alone, mepyramine and metiamide did not change CBF, heart rate, or blood pressure. Given in combination, these drugs decreased CBF slightly but did not change heart rate or blood pressure. Thus, changes in CBF response to histamine in the presence of the histamine antagonists singly or in combination are due to their blocking action and not to an independent action of the antagonists on the coronary vascular receptors. There was no attenuation in histamine responses during the course of an experiment, indicating absence of tachyphylaxis. Likewise, with the low doses of drugs, there was no evidence of deterioration of the preparation as reported by Parsons and Owen.

Dose ratios of histamine were compared at 50% of the maximum response found in the control dose-response curves. The dose ratios of histamine in the presence of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of Combined H₁ and H₂ Histamine Antagonists on Reactive Hyperemia (RH) following 8-Second Occlusions</th>
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<tbody>
<tr>
<td>RH (% repayment)</td>
<td>Peak flow (% increase)</td>
</tr>
<tr>
<td>Before histamine blockade</td>
<td>616 ± 120</td>
</tr>
<tr>
<td>After histamine blockade</td>
<td>620 ± 91</td>
</tr>
</tbody>
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Results (of five experiments in four dogs) are expressed as mean ± se.
HISTAMINE RECEPTORS IN THE CORONARY CIRCULATION/Giles et al.  

545

metiamide and mepyramine singly were 2 and 4, respectively. However, the combination of these histamine antagonists produced an augmented effect, the dose ratio increasing to 16. These results indicate that both H1 and H2 histamine receptors are present in the coronary vasculature and that blockade of the histamine response requires simultaneous administration of H1 and H2 receptor antagonists. H1 and H2 histamine receptors have been described in the peripheral vasculature of the dog. Histamine depressor responses have been reported in the cat and were partially blocked by mepyramine and abolished with the addition of burimamide or metiamide; yet H2 blocking drugs alone had no effect on the histamine depressor response.

Our results show that H1 receptor activity appears to predominate in the coronary vasculature. The doses of mepyramine and metiamide used gave near-maximal blocking effects without affecting resting CBF, heart rate, or blood pressure. The H1 and H2 receptors appear to have separate activities and histamine analogues have either H1 or H2 activity. The H1 receptor activity predominates and the augmented histamine blocking effect seen when mepyramine and metiamide are given in combination could be interpreted by either of the two models suggested by Powell and Brody. In the first model they suggest that histamine activates both H1 and H2 receptors, yet H1 activity predominates, which could be due to (1) a greater number of H1 receptors. (2) a greater affinity of histamine for H1 receptors, or (3) greater smooth muscle action as a result of H1 receptor activation. The second model suggests that histamine activates both H1 and H2 receptors equally, and if one of the group of receptors is blocked the action of the other is enhanced as a result of more histamine being made available to the remaining receptors. Our data do not indicate which of these possible interactions has occurred.

A physiological role for histamine in the regulation of blood flow has not been defined. Anrep and associates described the release of histamine-like substance from the dog heart and exercising human forearm and attributed reactive hyperemia to the production of histamine. Others were unable to confirm these findings and questioned the method used to detect histamine. In humans, Duff and associates found that H1 blocking drugs, including mepyramine, reduced reactive hyperemia following prolonged occlusions (10–15 minutes) of forearm blood flow, but had no effect on shorter occlusions (3–5 minutes). In the dog heart, we found that close coronary infusion of histamine produced vasodilation similar to that seen in reactive hyperemia following brief occlusions and that this histamine response could be abolished by H1 and H2 receptor antagonists given in combination. However, in the presence of both histamine antagonists there was no change in the reactive hyperemia. Folkow and associates found that the classical H1 histaminergic but not affect reactive hyperemia. They also suggested that there was a second histamine receptor that these histaminergic did not block. Our studies show that effective H1 and H2 receptor blockade does not change reactive hyperemia following brief coronary artery occlusions.

The use of intra-arterial histamine antagonists to identify histamine receptors assumes that these drugs are able to reach all such receptors. With intra-arterial histamine infusions, this appears to be so as the histamine effect on vascular receptors is blocked in the doses used. Moreover, increasing the doses of antagonists 10-fold did not alter the degree of blockade. We cannot exclude the possibility that what Dale has referred to as "intrinsic histamine" is released in response to brief occlusions and that the histamine antagonists are unable to block this effect. However, this is probably unlikely as it has been shown that histamine stimulates adenyl cyclase and produces cyclic AMP, effects which the H2 blocker burimamide competitively inhibits in both the intact heart and in homogenates of myocardium. Our data show there are H1 and H2 histamine receptors in the coronary vasculature of the dog and that histamine is a coronary vasodilator. The histamine receptors are competitively blocked with specific H1 and H2 antagonists and, when given together, each antagonist augments the effect of the other. Although the role of histamine in the regulation of coronary blood flow remains unknown, histamine activity does not appear to be a factor in reactive hyperemia following brief coronary artery occlusions.

Acknowledgments

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A Method for Determining Segmental Resistances in the Microcirculation from Pressure-Flow Measurements

STEPHEN H. NELLIS AND BENJAMIN W. ZWEIFACH

SUMMARY On the basis of an electrical analog, open circuit impedance functions were used to analyze the microcirculation. No specific structure need be assumed except a two-port, two-terminal network in which the major artery and vein supplying the tissue represent the input port and the two ends of the microvessel under study are the output port. The open circuit measurements were made by occluding microvessels in the exteriorized omentum of anesthetized rabbits. The pressure upstream and downstream to the occlusion defines the source pressure of a Thevenin's equivalent circuit. The equivalent resistance value was calculated by plotting the flow through a given microvessel against the pressure developed during a gradual occlusion. The changes in pressure vs. the changes in flow during a progressive occlusion were found to be linearly related. The Thevenin's equivalent resistance was maximum downstream to an occluded artery and upstream to the occluded vein. Within the capillary network, source pressures consistently were within a narrow range. Topically applied norepinephrine resulted in marked changes in source resistance and no changes in source pressures. Threshold doses of norepinephrine given intravenously resulted in changes in source pressures, but minimal changes in source resistance, even though a substantial change in vascular resistance was indicated when calculated on the basis of arterial pressure minus micropressure divided by microvessel flow. The present method defines the functional characteristics of the distributing vessels in terms of two pressures and two equivalent resistances and is relatively easy to perform. The technique can be used to determine the vascular components involved in the response to particular stimuli.

PRESSURE-FLOW relationships across discrete organs or masses of tissue usually are described by a lumped numerical expression representing the effective resistance encountered by the blood during its passage from artery to vein. A more precise description of the functional organization of the system would be feasible if the resistance in specific segments of the vascular tree could be measured. Pressure and flow interactions at the tissue level are difficult to formulate because of the complex structural organization of the microvascular network. An especially formable difficulty stems from the fact that discrete segments of the microcirculation are not supplied by a single source, nor are they drained by one vessel. In addition, the microvessels interconnect extensively at all levels of the system. Methods that do not involve a detailed analysis of all of the vascular elements must define alternative procedures for characterizing a network by a single peripheral resistance value. A possibility in this regard is to make certain a priori assumptions about the characteristics of the network bed. One such assumption is that it be represented by a simple parallel group of resistance vessels.

During the past few years, because of the capability of obtaining large numbers of direct measurements of microvascular indices, it has become possible to set up well documented distribution plots of both pressure and flow for discrete anatomical segments of a particular network. Such average values have been used to depict the spatial distribution of vascular resistance on the basis of idealized
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