**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 vasodilatation of reactive hyperemia after brief coronary artery occlusions does not appear to involve histamine.

**HISTAMINE**

acts through at least two specific receptors.¹ The development of H₁ receptor antagonists³ and more recently of specific H₂ receptor antagonists⁴ has made it possible to identify the presence in tissues of these two receptors. Histamine is taken up actively by the heart⁵ and its release may be quantified.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. Anesthesia 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.¹ 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 thoractomy, 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 FT245S) 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.
+2% full range deflection. Arterial pressures were measured with a Statham pressure transducer (model P23Db). Flow, pressure, and a standard lead I of the electrocardiogram were recorded with an Electronics for Medicine DR8 photographic recorder. The flow transducer was calibrated by using an appropriately sized artery and draining the dog's blood into a measuring cylinder over a 30-second period at the end of each experiment.

In two separate dogs a 5-cm-long, stiff-walled catheter (polyvinyl 14-gauge catheter) was introduced transmurally into the left ventricular cavity through the apex. This catheter was connected directly to a Statham P23Db pressure transducer. Alterations in left ventricular contractility were assessed from the peak rate of change of left ventricular pressure (dP/dt). The frequency response of the left ventricular recording system was flat ± 5% to 35 Hz using a sine wave pressure generator. Recordings were made at fast paper speed (200 mm/sec) for accurate analysis.

**PROTOCOL AND ANALYSIS OF RECORDS**

Histamine, metiamide, and mepyramine were given by close coronary arterial infusion through the polyvinyl catheter in the circumflex branch of the left coronary artery. Fresh solutions were prepared during each experiment in 0.9% saline and infused at 1 ml/min using a Braun constant infusion pump. Catheter transit time was assessed by recording the time taken for water, given at the usual infusion rate (1 ml/min), to displace air from the air-filled infusion catheter and its connections. Thus, although the period of infusion included the catheter transit time with all drugs, an estimate of the time for the response to histamine could be made once histamine had actually reached the coronary artery. Peak flow responses were measured and expressed as a percentage increase of the resting CBF. Histamine was infused repeatedly in three dogs to assess reproducibility of the histamine response.

Reactive hyperemic responses were obtained by rapidly inflating the pneumatic cuff to produce zero flow and then rapidly deflating it after 8 seconds. Changes in CBF, blood pressure, and heart rate were recorded until CBF had returned to preocclusion values.

Reactive hyperemia following 8-second occlusions and flow responses to drug infusions were recorded at a paper speed of 2.5 mm/sec with electrical means of CBF and pressure. The integral of the flow curve following reactive hyperemia was obtained by planimetry. Reactive hyperemic responses and flow responses to drug infusions were compared only if resting CBF, blood pressure, and heart rate values remained within ±10% throughout the experiment. At least 5 minutes were allowed between each occlusion or infusion to allow complete recovery of the response. Flow debt, reactive hyperemic flow, and percent repayment of flow debt were calculated as described by Coffman and Gregg according to the following formula:

Flow debt = control flow rate × duration of occlusion

Reactive hyperemic flow

= (integral of the flow curve during reactive hyperemia)

− (control flow rate × duration of reactive hyperemia)

Percent repayment of flow debt

= reactive hyperemic flow/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.

**METIAMIDE AND MEPYRAMINE**

In preliminary experiments, mepyramine (100 μg/min) or metiamide (100 μg/min) given separately by intracorono-
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.

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.
amide (100 μg/min). CBF = coronary blood flow. Values are

probably represents maximal coronary vasodilation. By

due to coronary arterial catecholamine. We were able to give low
dose infusions which reached the vascular receptors with-
out producing systemic effects. In the two dogs in which
contractility changes were assessed, there was no altera-
tion in contractility to the dose of histamine used by itself
or in combination with the histamine antagonists. Thus,
CBF responses were largely due to the direct action of the
infused drugs and not to metabolic changes secondary to
contractility changes.

Powell and Brody\textsuperscript{14} recently described positive chrono-
tropic and negative inotropic cardiac responses to intrave-
nous 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
pacing 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 ap-
proximately 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\textsuperscript{15} 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 inhibi-
tion. 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 histo-
amine 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. Like-
wise, with the low doses of drugs, there was no evidence of
deterioration of the preparation as reported by Parsons
and Owen.\textsuperscript{16}

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

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
RH & Peaß flow & Duration & Heart rate & Blood pressure \\
(% repayment) & (% increase) & of RH (sec) & (beat/min) & (mm Hg) \\
\hline
Before histamine blockade & 616 ± 120 & 175 ± 27 & 87 ± 9 & 171 ± 9 & 144 ± 3 \\
After histamine blockade & 620 ± 91 & 177 ± 19 & 83 ± 11 & 171 ± 9 & 142 ± 2 \\
\hline
\end{tabular}
\caption{Effect of Combined H\textsubscript{1} and H\textsubscript{2} Histamine Antagonists on Reactive Hyperemia (RH) following 8-Second Occlusions}
\end{table}

Results (of five experiments in four dogs) are expressed as mean ± se.
Histamine receptors in the coronary circulation

Giles et al.

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.18,19

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.1,2 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.17 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 associates15,20 described the release of histamine-like substance from the dog heart and exercising human forearm and attributed reactive hyperemia to the production of histamine. Others21-25 were unable to confirm these findings and questioned the method used to detect histamine. In humans, Duff and associates26 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 associates27 found that the classical 
H1 antihistamines did not affect reactive hyperemia. They also suggested that there was a second histamine receptor that these antihistamines 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 Dale26 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 adenylyl cyclase and produces cyclic AMP effects which the 
H2 blocker burimamide competitively inhibits in both the intact heart27 and in homogenates of myocardium.58

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

We thank Patrick Legge for technical assistance and Pat Wilde for secretarial assistance.

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H2-receptor
A Method for Determining Segmental Resistances in the Microcirculation from Pressure-Flow Measurements

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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 Thévenin'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 Thévenin'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.1 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.2 An especially formidable 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.3 Such average values have been used to depict the spatial distribution of vascular resistance on the basis of idealized...
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Circ Res. 1977;40:541-546
doi: 10.1161/01.RES.40.6.541

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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