The Interrelationship among Histamine, Various Vasoactive Substances, and Macromolecular Permeability in the Canine Forelimb

George J. Grega, James J. Maciejko, Richard M. Raymond, and Daniel P. Sak

SUMMARY The local intra-arterial infusion of histamine, in doses which produce maximal increases in protein efflux, failed to increase lymph total protein concentration in canine forelimbs perfused at constant inflow with autologous blood from dogs subjected to severe hemorrhagic hypotension. Norepinephrine and isoproterenol in low concentrations were less effective in antagonizing protein efflux produced by high doses of histamine than by low doses of this agent. This suggests that other vasoactive substances released in response to a hemorrhagic stimulus may also be physiological antagonists of the direct actions of histamine on the microvascular membrane. The simultaneous infusion of vasopressin or serotonin, or pretreatment with high doses of glucocorticoids, antagonizes the protein efflux produced by local intra-arterial infusions of histamine into forelimbs perfused at constant inflow. Angiotensin II, acetylcholine, low doses of glucocorticoids, and papaverine all failed to alter measurably the protein efflux produced by histamine under similar conditions. We conclude that a variety of hormones and other vasoactive agents may function as antagonists of the direct action of histamine on the microvascular membrane, and that the antagonism of histamine-induced protein efflux is independent of changes in histamine receptor blockade, blood flow, microvascular pressure, and perfused surface area. Moreover, in experiments employing adrenergic blocking agents, the antagonism of histamine-induced protein efflux by catecholamines was due to stimulation of $\beta$-adrenergic receptors. Circ Res 46: 264-275, 1980

It has recently been demonstrated that catecholamines antagonize the protein efflux produced by local intra-arterial infusions of histamine (4 $\mu$g base/min) into canine forelimbs perfused either naturally or at constant inflow (Marciniak et al., 1978). This antagonism of histamine-induced protein efflux by a direct action of catecholamines on the microvascular membrane is independent of histamine receptor blockade and of changes in microvascular pressure, perfused surface area, and total blood flow (Marciniak et al., 1978; Maciejko et al., 1978; Rippe and Grega, 1978). A previous study reported that severe hemorrhagic hypotension almost completely antagonized the protein efflux produced by both submaximal (4 $\mu$g base/min) and maximal (64 $\mu$g base/min) doses of histamine infused locally intra-arterially into forelimbs perfused at constant inflow (Marciniak et al., 1977). The dose of histamine producing maximal increases in protein efflux rapidly increases lymph protein concentration to values approaching that in plasma and produces massive increases in forelimb weight in 20 minutes or less (Haddy et al., 1972; Grega et al., 1972a). These very dramatic increases in weight and protein efflux fail to develop if the same dose of histamine is infused intra-arterially into forelimbs perfused with blood from animals subjected to severe hemorrhagic hypotension. Although perfusion of the forelimb with blood from animals subjected to severe blood loss prevents the histamine-induced increases in protein efflux and edema formation, histamine still causes profound vasodilation in the forelimb. It was suggested (Marciniak et al., 1977, 1978) that the failure of histamine to increase protein efflux and produce edema under these conditions might be attributable to an endogenous release of catecholamines (and perhaps other substances) subsequent to hypotension and the direct action of histamine on the adrenal medulla.

One aim of the present study was to determine if catecholamines in blood from animals subjected to severe arterial blood loss are solely responsible for the antagonism of the protein efflux produced by both submaximal and maximal doses of histamine, or if other naturally occurring vasoactive agents liberated into blood in response to the bleeding stimulus also function as antagonists of the direct actions of histamine on the microvascular membrane. The effects of several synthetic vasoactive substances on histamine-induced increases in protein efflux were also studied for comparison pur-
poses. A second aim was to confirm the suggestion (Marciniak et al., 1977, 1978) that the antagonism of the histamine-induced increases in protein efflux is due to a stimulation of β-adrenergic receptors.

**Methods**

Mongrel dogs of either sex having an average weight of 24 kg (16- to 29-kg range) were anesthetized with sodium pentobarbital (30–35 mg/kg, iv) and ventilated with room air, using a Harvard respirator.

**Lymph Flow Rate and Total Protein Concentration Measurements**

Small incisions were made over the brachial artery, cephalic vein (above the elbow), and second superficial dorsal metacarpal vein (paw) in the right forelimb. A side branch of the brachial artery, a lymph vessel, and the vein were isolated. After administering heparin intravenously (500 units/kg), we cannulated these vessels in an upstream direction with polyethylene tubing. The cannulas were used for drug administration, lymph collection, and pressure measurement, respectively. The lymph vessels in the area of the cephalic vein at the elbow drain forelimb skin and paw (Haddy et al., 1972). Two or three lymph vessels were usually tied centrally, and one of them was cannulated distally with a 10-cm length of PE-10 tubing that had been beveled at the cannulating end.

Lymph was collected in miniature 0.5-ml graduated cylinders constructed from plastic pipettes. Drugs were infused into the brachial artery with a Harvard infusion pump. The skin small vein pressure and aortic pressure (via a femoral artery) were measured with low-volume displacement Statham pressure transducers and recorded on a Hewlett-Packard direct-writing oscillograph. The brachial and cephalic veins. The forelimb nerves (median, ulnar, radial, and musculocutaneous) were left intact and coated with an inert silicone spray to prevent drying. Heparin was administered intravenously to prevent clotting.

Intravascular pressures were measured in the brachial and cephalic veins with small-bore polyethylene catheters inserted via side branches. The second superficial dorsal metacarpal vein was cannulated in an upstream direction to measure skin small vein pressure. Pressures were measured with low-volume displacement Statham P23Gb transducers and recorded on a Hewlett-Packard direct-writing oscillograph. The brachial and cephalic veins were partially transected 3–5 cm downstream from the sites of the large vein pressure catheters, and the end of each vessel was cannulated with a short section of PE-320 tubing. The outflow from both veins was directed into a reservoir maintained at constant volume with a variable speed Holter pump that continuously returned blood to the dog via a cannulated jugular vein. Blood flow was determined by timed collections of the two venous outflows. The median cubital vein, which represents the major anastomotic connection between the brachial and cephalic veins, was ligated in all ex-
periments so that brachial venous flow was predominately from muscle, whereas cephalic venous flow was predominately from skin. Although this approach does not provide complete anatomical separation of skin and muscle, the degree of flow separation is sufficient to permit comparison of resistance changes in these two parallel-coupled beds (Grega and Haddy, 1971; Grega et al., 1971, 1972a; 1972b). In some experiments, forelimb inflow was held constant throughout the experiment at a rate which initially produced a perfusion pressure similar to systemic pressure. This was accomplished by pumping femoral arterial blood into the brachial artery with a Sigmamotor pump. Perfusion pressure was monitored continuously via a catheter inserted into a side branch of the brachial artery.

When all cannulas were in position, the limb was suspended on a wire mesh platform attached to a sensitive strain gauge I-beam balance which could be calibrated by adding known weights to the platform. The addition of a 2-g weight usually produced a pen deflection of 10–20 mm. Mean systemic arterial pressure was measured continuously from a catheter in the lower abdominal aorta. Total skin and muscle resistances and skin large vein resistance were calculated by dividing brachial, cephalic, or total forelimb blood flows into corresponding pressure gradients. Histamine (64 µg base/min) was infused locally intra-arterially for 60 minutes, either alone or with isoproterenol (4 µg/min), in forelimbs perfused both naturally and at constant inflow.

All data were analyzed statistically by analysis of variance (randomized complete block design). The means were compared by the least significant difference test (Sokal and Rohlf, 1969).

**Results**

**Lymph: Effects of Catecholamines and Histamine (Table 1)**

**Natural Flow**

In naturally perfused forelimbs, the local intra-arterial infusion of histamine (64 µg base/min) for 60 minutes produced marked increases in skin lymph flow and lymph protein concentration, and the forelimbs became rapidly and visibly edematous. Systemic pressure was substantially decreased, whereas skin small vein pressure was markedly increased.

The simultaneous 60-minute isoproterenol (4 µg/min, ia)-histamine infusion produced an increase in lymph flow and lymph protein concentration. However, the increase in lymph flow and lymph protein concentration was far less than that produced by histamine alone. Systemic pressure was decreased and skin small vein pressure was increased during the infusion period.

The simultaneous 60-minute norepinephrine-histamine infusion (4 µg base/min, ia) caused only a very slight increase in lymph flow rate. Lymph protein concentration was increased, but the increase was far less than that produced by histamine alone. Systemic pressure was increased transiently, and skin small vein pressure was increased during the infusion period.

**Constant Flow**

In forelimbs perfused at constant inflow, histamine (64 µg base/min, ia) infused for 60 minutes still produced marked increases in skin lymph flow and lymph protein concentration. The increase in lymph flow and lymph protein concentration was, however, less than that observed during the infusion of corresponding doses into naturally perfused forelimbs. Systemic pressure decreased, whereas skin small vein pressure was unchanged relative to control. Perfusion pressure was significantly reduced during the infusion period.

The 60-minute infusion of isoproterenol (4 µg/min, ia) with the high dose of histamine increased skin lymph flow and lymph protein concentration, but the increase was far less than that produced by histamine alone. Systemic pressure and perfusion pressure were decreased, whereas skin small vein pressure was increased transiently during the infusion period.

The 60-minute norepinephrine (16 µg base/min, ia) -histamine infusion caused a small increase in lymph flow and lymph protein concentration. However, lymph protein concentration was increased only transiently relative to that usually produced by this dose of histamine alone. The combined infusion produced only a small, transient increase in systemic pressure, but produced very marked increases in perfusion pressure and skin small vein pressure.

**Weight: Effects of Histamine and Isoproterenol (Table 2)**

**Natural Flow**

In naturally perfused forelimbs, histamine (64 µg base/min, ia) infused for 60 minutes produced massive increases in forelimb weight, skin and skeletal muscle blood flows, and skin small vein pressure. Skin large vein resistance and systemic pressure decreased relative to control.

During the combined histamine-isoproterenol (4 µg/min, ia) infusion, skin and skeletal muscle blood flows and skin small vein pressure still were markedly increased, and systemic pressure still was moderately decreased. There was no significant increase in skin large vein resistance during the combined infusion. However, the increase in forelimb weight was roughly one-third of that elicited by histamine alone.

**Constant Flow**

In the forelimbs perfused at constant inflow, histamine (64 µg base/min, ia) infused for 60 minutes produced marked increases in forelimb weight and
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<th>Infusion period (min)</th>
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### Systemic arterial pressure (mm Hg)

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<th>H64 ISO4</th>
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<tr>
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### Lymph total protein concentration (g/100 ml)

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### Perfusion pressure (mm Hg)

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### Skin small vein pressure (mm Hg)

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### Lymph flow (ml/10 min)

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### Lymph total protein concentration (g/100 ml)

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<tr>
<th></th>
<th>NF H64</th>
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<td>±0.0 ± 0.2*</td>
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### Control lymph total protein concentration (g/100 ml)

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### NF = natural flow, CF = constant flow; H64 = histamine, 64 μg base/min, ia; ISO4 = isoproterenol, 4 μg base/min, ia; NE4 = norepinephrine, 4 μg base/min, ia; NE16 = norepinephrine, 16 μg base/min, ia.

Control lymph total protein concentration usually ranged between 40 and 55% of plasma total protein concentration. Plasma total protein concentration failed to change during the infusion period from the control value. Results in this and all tables are expressed as mean ± SE.

* P < 0.01; †P < 0.05.
TABLE 2  Effects of High Concentrations of Histamine and Low Concentrations of Isoproterenol on Weight and Hemodynamics in Forelimbs Perfused either Naturally or at Constant Inflow (n = 6 for all experiments).

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<thead>
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<td>Δ Weight (g)</td>
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<td>ISO4</td>
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<td>CF</td>
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<td>Cephalic venous outflow (ml/100 g)</td>
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NF = natural flow; CF = constant flow; H64 = histamine, 64 µg base/min, ia; ISO4 = isoproterenol, 4 µg/min.

* P < 0.01; †P < 0.05.

decreases in perfusion pressure, but less than that under natural flow conditions. There was no significant shift in blood flow between skin and skeletal muscle, and skin small vein pressure and skin large vein resistance were unaltered relative to control.

During the combined histamine-isoproterenol (4 µg/min, ia) infusion, there was no significant change in any of the variables relative to that produced by histamine alone except forelimb weight. Weight increased only one-fourth to one-fifth of the amount produced by the infusion of histamine alone.

Other Vasoactive Agents Infused with Histamine (Tables 3, 4, and 5)

With the exception of histamine, none of the other vasoactive agents studied measurably altered lymph total protein concentration. However, all vasoactive agents studied had marked effects on forelimb vascular resistance with the exception of methylprednisolone. This agent failed to measurably alter forelimb vascular pressures.

The local intra-arterial infusion of acetylcholine (10 µg/min), angiotensin II (2 µg/min), methylprednisolone (50 µg/min), and papaverine (0.4 mg/min) with histamine (4 µg base/min, ia) for 60 minutes elicited a large increase in lymph protein concentration similar to that produced by histamine alone. Vasopressin (0.6 PU/min) or serotonin (15 µg base/min, ia) infused locally intra-arterially with histamine (4 µg base/min) largely prevented the increase in lymph protein concentration. The intravenous administration of methylprednisolone (30 mg/kg) 20 minutes prior to the local intra-arterial infusion of histamine also prevented the histamine-induced increase in lymph protein concentration. Perfusion pressure was decreased and skin small vein pressure failed to change relative to control during the infusion of acetylcholine, papaverine, and methylprednisolone with histamine. Perfusion pressure was unchanged, whereas skin small vein pressure was increased during the vasopressin, serotonin, and angiotensin II infusions with histamine.
whereas isoproterenol decreased perfusion pressure
flow and total protein concentration relative to
mine (4 μg base/min) infused locally intra-arterially

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<td>Acetylcholine (n = 6)</td>
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<td>Perfusion pressure (mm Hg)</td>
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<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.1 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin II (n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>109 ± 5</td>
<td>191 ± 7*</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>11 ± 1</td>
<td>15 ± 2*</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.4 ± 0.3</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>Papaverine (n = 5)</td>
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<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>110 ± 4</td>
<td>70 ± 6*</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>10 ± 1</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.02 ± 0</td>
<td>0.02 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylprednisolone (n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>120 ± 6</td>
<td>119 ± 7</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.5 ± 0.3</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylprednisolone bolus (n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>112 ± 3</td>
<td>112 ± 3</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.25 ± 0.2*</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.4 ± 0.3</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin (n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>120 ± 4</td>
<td>119 ± 4</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.20 ± 0.2*</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.4 ± 0.3</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vasopressin (n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>112 ± 3</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>10 ± 1</td>
<td>14 ± 2*</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.3 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
</tbody>
</table>

Acetylcholine: 10 μg/min, ia; Angiotensin II: 2 μg/min, ia; Papaverine: 0.4 μg/min, ia; Methylprednisolone: 50 μg/min, ia; Methylprednisolone bolus: 30 mg/kg, iv; Serotonin: 15 μg base/min, ia; Vasopressin: 0.6 P.U./min, ia.

Control lymph total protein concentration usually ranged between 40 and 55% of plasma total protein concentration (PPC). PPC failed to change significantly during the infusion period relative to control.

* P < 0.01; ** P < 0.005.

Adrenergic Blockade Catecholamines, and Histamine (Table 6)

In forelimbs perfused at constant inflow, histamine (4 μg base/min) infused locally intra-arterially for 60 minutes caused a sustained reduction in perfusion pressure and marked increases in lymph flow and total protein concentration. Small skin vein pressure failed to change relative to control. Thirty-minute local intra-arterial infusions of nor-epinephrine or isoproterenol failed to alter lymph flow and total protein concentration relative to control. Nor-epinephrine elicited marked increases in perfusion pressure and skin small vein pressure, whereas isoproterenol decreased perfusion pressure but failed to alter skin small vein pressure. After pretreatment with phenolamine, lymph flow and total protein concentration failed to increase during the 60-minute infusion of histamine (4 μg base/min, ia) with either isoproterenol (3 μg/min, ia) or nor-epinephrine (4 μg base/min, ia). Following α-adrenergic blockade, perfusion pressure still fell, and skin small vein pressure still failed to change relative to control during the histamine-isoproterenol infusion. In contrast, phenolamine pretreatment prevented the increase in perfusion pressure and skin small vein pressure during the histamine-nor-epinephrine infusion. In fact, perfusion pressure decreased to levels similar to that produced by histamine alone.
After pretreatment with propranolol, the combined histamine-norepinephrine or isoproterenol infusion elicited increases in lymph flow rate and lymph total protein concentration. The increase in lymph total protein concentration was similar in magnitude to that produced by the infusion of histamine alone. Pretreatment with propranolol failed to prevent the decrease in perfusion pressure during the histamine-isoproterenol infusion or to prevent the increase in perfusion pressure and skin small vein pressure during the histamine-norepinephrine infusion.

Discussion

It has been reported previously that, in canine forelimbs perfused either naturally or at constant inflow, the simultaneous local intra-arterial infusion of norepinephrine (4 µg base/min) or isoproterenol (3 µg/min) completely prevents (Marciniak et al., 1978) the increase in lymph protein concentration produced by submaximal concentrations of histamine (4 µg base/min). In this study, it was noted that these concentrations of isoproterenol and norepinephrine do not prevent completely the increase in lymph protein concentration produced by higher doses of histamine (64 µg base/min, ia). However, the initial increase in lymph protein concentration was less than usual, and within 1 hour, lymph protein concentration decreased to near control levels. In contrast, this same high dose of histamine infused alone intra-arterially for 60 minutes produced very marked, sustained increases in lymph protein concentration.

The antagonism of the histamine-induced pro-

### Table 4 Effects of Histamine Infused with Vasactive Agents which Failed to Prevent the Increase in Lymph Protein Concentration in Canine Forelimbs Perfused at Constant Inflow

<table>
<thead>
<tr>
<th></th>
<th>Control (min)</th>
<th>Infusion period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-20 to 10</td>
<td>-10 to 0</td>
</tr>
<tr>
<td><strong>Histamine-acetylcholine infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>106 ±5 106</td>
<td>52 ±4 57 ±4 57 ±3</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>13 ±1 13</td>
<td>13 ±1 12 ±1 12 ±1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ±0.01</td>
<td>0.04 ±0.18 0.20 ±0.23</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>1.8 ±0.2 1.8</td>
<td>2.4 ±0.3 2.8 ±0.3 3.4 ±0.3 3.2 ±0.3</td>
</tr>
<tr>
<td><strong>Histamine-angiotensin II infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>110 ±3 110</td>
<td>120 ±4 90 ±3 75 ±3 72 ±3 72 ±3 75 ±3 75 ±3 75 ±3</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>10 ±1 10</td>
<td>11 ±1 14 ±1 14 ±1 13 ±1 12 ±1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ±0.01</td>
<td>0.21 ±0.41 0.55 ±0.53 0.52 ±0.44</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>1.6 ±0.1 1.6</td>
<td>2.5 ±0.3 3.3 ±0.3 3.9 ±0.3 3.7 ±0.3 3.4 ±0.3</td>
</tr>
<tr>
<td><strong>Histamine-papaverine infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>118 ±2 118</td>
<td>60 ±4 63 ±4 63 ±5 65 ±5 66 ±5 67 ±5</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>11 ±1 11</td>
<td>11 ±1 11 ±1 11 ±1 11 ±1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ±0.01</td>
<td>0.23 ±0.37 0.32 ±0.33 0.29 ±0.17 0.27 ±0.14</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.2 ±0.2 2.2</td>
<td>3.0 ±0.3 4.4 ±0.3 4.1 ±0.3 4.2 ±0.3</td>
</tr>
<tr>
<td><strong>Histamine-methylprednisolone infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>120 ±2 120</td>
<td>54 ±4 56 ±4 56 ±3 57 ±3 57 ±3 57 ±3 57 ±3 57 ±3</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>12 ±1 12</td>
<td>12 ±1 12 ±1 12 ±1 12 ±1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ±0.01</td>
<td>0.18 ±0.41 0.41 ±0.36 0.28 ±0.25</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>1.7 ±0.2 1.7</td>
<td>2.3 ±0.2 2.9 ±0.2 3.4 ±0.2 3.5 ±0.2</td>
</tr>
</tbody>
</table>

Histamine, 4 µg base/min, ia; Acetylcholine, 10 µg/min, ia; Angiotensin II, 2 µg/min, ia; Papaverine, 0.4 mg/min, ia; Methylprednisolone, 50 µg/min, ia.

Control lymph total protein concentration usually ranged between 40 and 50% of plasma total protein concentration. Plasma total protein concentration failed to change significantly during the infusion period relative to control.

* P < 0.01; †P < 0.05.
protein efflux produced by low doses of catecholamines is independent of changes in blood flow, perfused surface area, and microvascular pressure. For example, small vein pressure (and, inferentially, microvascular pressure) is very markedly increased during the norepinephrine-histamine infusion relative to that during the infusion of histamine alone, yet the increase in lymph flow and lymph protein concentration was considerably less during the histamine-norepinephrine infusion. This is also supported by the weight and hemodynamic data. The massive edema formation produced by this high dose of histamine (64 μg base/min) was reduced greatly by the simultaneous infusion of isoproterenol in forelimbs perfused either naturally or at constant inflow owing to the marked inhibition of the protein efflux. The reduction in edema formation by isoproterenol was less in the forelimbs perfused at natural inflow. This is attributable to the marked increase in small vein pressure (and, inferentially, in microvascular pressure) and perfused surface area subsequent to arteriolar dilation under natural flow conditions. In contrast, in the forelimbs perfused at constant inflow, skin small vein pressure failed to increase relative to control as the blood pump mechanically prevented the increase in blood flow.

A fall in blood flow cannot have contributed to the antagonism of the histamine-induced protein efflux produced by isoproterenol. Both skin and skeletal muscle blood flows were increased markedly under natural flow conditions, and there was no significant shift in blood flow between skin and skeletal muscle in the constant flow experiments. This conclusion is also supported by the findings of Rippe and Grega (1978). In the maximally dilated rat hindquarter preparation, the simultaneous infusion of histamine and isoproterenol completely prevented the marked increase in filtration and the capillary filtration coefficient (CFC) produced by histamine. The permeability-surface area product (PS) for Cr-EDTA failed to change during the simultaneous histamine-isoproterenol infusion relative to control; hence, there was no reduction in perfused surface area. It also was observed in this preparation that the marked increase in fluid filtration and CFC produced by histamine could be dramatically reduced by the infusion of isoproterenol given after the start of the histamine infusion. Therefore, it must be concluded that the antagonism of histamine-

<table>
<thead>
<tr>
<th>TABLE 5 Effects of Histamine Infused with or after the Injection of Agents which Largely Prevented the Increase in Lymph Total Protein Concentration in Canine Forelimbs Perfused at Constant Inflow.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (min)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Histamine-vasopressin infusion (n = 6)</td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Histamine-serotonin infusion (n = 6)</td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Methylprednisolone bolus (n = 6)</td>
</tr>
</tbody>
</table>

Histamine, 4 μg base/min, ia; Vasopressin, 0.6 pressor units/min, ia; Serotonin, 15 μg base/min, ia; Methylprednisolone, 30 mg/kg, iv.

\*P < 0.01; \*P < 0.05.
TABLE 6  Effects of Adrenergic Receptor Blockade on Hemodynamic and Lymph Parameters during the Histamine-Catecholamine Infusions into Forelimbs Perfused at Constant Inflow.

<table>
<thead>
<tr>
<th></th>
<th>Control period</th>
<th>Infusion period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-20 to -10</td>
<td>-10 to 0</td>
</tr>
<tr>
<td><strong>Histamine infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>114 ± 4</td>
<td>118 ± 4</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>10 ± 1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.2 ± 0.1</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td><strong>Norepinephrine infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>120 ± 5</td>
<td>119 ± 5</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>10 ± 1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.02 ± 0</td>
<td>0.02 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td><strong>Isoproterenol infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>113 ± 4</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.4 ± 0.3</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td><strong>After phenolamine</strong></td>
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<tr>
<td><strong>Histamine infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>120 ± 5</td>
<td>120 ± 5</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.1 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td><strong>Histamine-isoproterenol infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>112 ± 4</td>
<td>110 ± 4</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.02 ± 0</td>
<td>0.02 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td><strong>Histamine-norepinephrine infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>110 ± 3</td>
<td>110 ± 3</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>10 ± 1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
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<td><strong>After propranolol</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>Histamine infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>107 ± 4</td>
<td>107 ± 4</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td><strong>Histamine-isoproterenol infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>103 ± 3</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.2 ± 0.3</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td><strong>Histamine-norepinephrine infusion (n = 6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure (mm Hg)</td>
<td>105 ± 4</td>
<td>106 ± 4</td>
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<tr>
<td>Skin small vein pressure (mm Hg)</td>
<td>10 ± 1</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Lymph flow (ml/10 min)</td>
<td>0.01 ± 0</td>
<td>0.01 ± 0</td>
</tr>
<tr>
<td>Lymph total protein concentration (g/100 ml)</td>
<td>2.1 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
</tbody>
</table>

Histamine, 4 μg base/min, in. Isoproterenol, 3 μg/min, in. Norepinephrine, 4 μg base/min, in. Control lymph total protein concentration usually ranged between 20 and 55% of plasma total protein concentration. Plasma total protein concentration failed to change during the infusion period relative to control.

* P < 0.01; † P < 0.05.
mine-induced protein efflux produced by isoproterenol is attributable to a direct action on the microvascular membrane which effectively counteracts the effects produced by histamine.

This conclusion is supported by morphological data that demonstrate that the direct action of histamine and bradykinin on the microvascular membrane causes the formation of a large number of venular gaps (Casley-Smith and Bolton, 1973; Casley-Smith and Window, 1976; Hurley, 1972; Haddy et al., 1976). These gaps are up to 1 μm in diameter but remain maximally open for only about 30 minutes. The large venular gaps are said to be created by a "rounding up" of the ends of adjacent endothelial cells due to a contraction of actomyosin-like anchoring filaments. Therefore, it is assumed that catecholamines, by stimulation of β-adrenergic receptors, cause a relaxation of these contractile filaments and thereby physiologically counteract the direct action of histamine on the microvascular membrane. This support is by intravital microscopic observations: the marked bradykinin- and histamine-induced increase in the number of venular leakage sites of fluorescein-dextran (mol wt 145,000) in the hamster cheek pouch is greatly reduced by the simultaneous application of catecholamines (Svensjo et al., 1977). The fact that cooling largely prevents the increase in macromolecular efflux produced by histamine also suggests that the formation of venular gaps involves an active contractile process (Rippe and Grega, 1978).

These data demonstrate that the effectiveness of autologous blood from animals subjected to severe hemorrhagic hypotension in antagonizing the protein efflux produced by even high concentrations of histamine is attributable, in part, to some noncatecholamine vasoactive substance(s). This is suggested because catecholamines only partially antagonize the protein efflux produced by high concentrations of histamine. Therefore, a series of experiments was designed to determine if other naturally occurring or synthetic vasoactive agents had the ability to antagonize the protein efflux produced by a dose of histamine which caused marked but submaximal increases in lymph total protein concentration. The doses of the other vasoactive agents studied were selected to produce marked effects on forelimb vascular pressures, but minimal or small systemic effects. The large dose of methylprednisolone was based on that reported in the literature as exerting beneficial effects in the treatment of experimental circulatory shock states. All vasoactive agents except methylprednisolone had marked effects on forelimb vascular pressures when infused alone into forelimbs perfused at constant inflow. Neither the bolus injection nor the local intraarterial infusion of methylprednisolone produced measurable effects on forelimb vascular pressures. Most importantly, none of the other vasoactive agents employed in this part of the study had measurable effects on lymph total protein concentration during the 60-minute observation period.

The data from this study demonstrate that several other substances released in response to hemorrhage also antagonize the protein efflux produced by histamine (4 μg base/min) infused locally intrarterially into forelimbs perfused at constant inflow. Vasopressin, serotonin, and high pharmacological doses of glucocorticoids counteracted the direct action of histamine on the microvascular membrane and antagonized the protein efflux. Although these agents prevented the increase in lymph total protein concentration produced by this dose of histamine, the vasodilator action of histamine still was evident. However, angiotensin II, acetylcholine, the synthetic vasodepressor papaverine, and the low dose of methylprednisolone were without measurable effect on histamine-induced protein efflux. As with the catecholamines, the antagonism of the histamine-induced protein efflux by vasopressin, serotonin, and high pharmacological doses of glucocorticoids must be attributed to a direct action of these agents on the microvascular membrane. For example, high concentrations of methylprednisolone had no measurable vascular effects and failed to prevent the marked reduction in perfusion pressure produced by histamine. However, it completely prevented the increase in lymph protein concentration normally produced by histamine. Angiotensin II in concentrations that produce profound vasoconstriction failed to prevent the increase in lymph protein concentration produced by histamine. Vasopressin in concentrations producing pressor effects equal to those of angiotensin II completely prevented the increase in lymph protein concentration produced by histamine. In contrast to the vasodilator catecholamine isoproterenol, the vasodilators acetylcholine and papaverine failed to prevent the increase in lymph protein concentration produced by histamine. It is evident that this antagonism of histamine-induced protein efflux cannot be explained by changes in blood flow or microvascular pressure and, therefore, must represent a direct action of these agents on the microvascular membrane which counteracts that of histamine. It is interesting to note that vasopressin, which also is the antidiuretic hormone, affects permeability in the distal tubule and collecting duct of the nephron. Also, of interest is the fact that serotonin increases microvascular permeability to macromolecules in rodents; however, in dogs and humans, serotonin fails to alter macromolecular permeability (Haddy et al., 1976). The failure of a low concentration of glucocorticoids to antagonize histamine-induced protein efflux suggests that physiological concentrations of naturally occurring glucocorticoids probably do not prevent increases in microvascular permeability to macromolecules produced by histamine. Thus, a number of hormones may function as physiological antagonists of the direct actions of histamine on the microvascular membrane. Increases in microvascular permeability produced by histamine and related substances in various pathophysiological (e.g., inflammatory and anaphylactic
reactions) states may be limited by increases in the concentration of one or more of these hormones. In contrast to the small pore system, the venular large pore system is subject to physiological and pharmacological control.

The antagonism of histamine-induced increases in protein efflux produced by the catecholamines is due to the stimulation of $\beta$-adrenergic receptors. Pretreatment with phentolamine failed to alter the antagonism of the histamine-induced protein efflux produced by either isoproterenol or norepinephrine in forelimbs perfused at constant inflow suggesting no involvement of $\alpha$-adrenergic receptors. Phentolamine prevented the increase in perfusion pressure and skin small vein pressure during the norepinephrine-histamine infusion, yet failed to prevent the antagonism of the histamine-induced protein efflux produced by norepinephrine. Propranolol pretreatment prevented the antagonism of the histamine-induced protein efflux produced by both catecholamines in forelimbs perfused at constant inflow indicating that the antagonism is mediated via stimulation of $\beta$-adrenergic receptors. The increase in lymph total protein concentration produced by the histamine-catecholamine infusion after pretreatment with propranolol was similar to that produced by the infusion of histamine alone.

It is interesting to note that the histamine dose-response relationship for vasodilation differs from that for increasing macromolecular permeability (Grega et al., 1972a; Haddy et al., 1976). The local intra-arterial infusion of 1 $\mu$g base/min produces nearly maximal vasodilation. In contrast, it takes an infusion of 1.5–2 $\mu$g base/min of histamine to cause consistent increases in protein efflux and 40–60 $\mu$g base/min to produce nearly maximal increases in protein efflux. This may be important physiologically because low concentrations of histamine could play a role in the local regulation of blood flow without increasing microvascular permeability to plasma proteins. However, in pathophysiological states such as anaphylactic and inflammatory reactions, a massive local endogenous release of histamine or other edemogenic agents could alter macromolecular permeability profoundly, resulting in massive edema formation as is often seen clinically.

These data suggest that the existing physiological state may greatly modify the microvascular response to histamine via changes in mean aortic pressure. For example, local intra-arterial infusions of histamine (4–64 $\mu$g base/min) into canine forelimbs perfused at constant inflow induces marked dose-related increases in protein efflux and edema formation (Grega et al., 1972a; Marciniak et al., 1978; Haddy et al., 1976). Mean aortic pressure either fails to change relative to control or, with the highest dose of histamine, decreases from a control of roughly 120 mm Hg to 95 mm Hg by the end of the infusion period. Moreover, the decrease in mean aortic pressure produced by the high dose of histamine usually is not prominent until after the edema has largely developed. Local intra-arterial infusions of histamine produce edema even if forelimb perfusion pressure is locally decreased to 30 mm Hg by use of a blood pump (Grega et al., 1972a). In contrast, if mean aortic pressure is pharmacologically decreased to approximately 70 mm Hg prior to initiating the local intra-arterial infusion of histamine (Marciniak et al., 1977), then the ensuing histamine-induced protein efflux either is largely prevented or is considerably reduced, depending on the dose studied, even if forelimb blood flow is maintained constant at control levels. If mean aortic pressure is decreased to approximately 40 mm Hg by arterial hemorrhage prior to initiating the local intra-arterial infusion of histamine into forelimbs perfused at constant inflow at control flow rates, the histamine-induced protein efflux produced by both low and high doses of histamine is almost completely prevented. Thus, the more marked the reduction in mean aortic pressure, the less the increase in protein efflux produced by a given dose of histamine infused locally intra-arterially into forelimbs perfused at constant inflow at control flow rates. This reduced responsiveness of the microvascular membrane to histamine is probably attributable to the release of vasoactive agents subsequent to the degree of systemic hypotension. A reduction in mean aortic pressure to 70 mm Hg will cause a reflex release of catecholamines from adrenergic nerve endings and from the adrenal medulla. A still further reduction in mean aortic pressure to 40 mm Hg will intensify the release of catecholamines and increase the plasma levels of a variety of other agents, including angiotensin II, vasopressin, serotonin, and glucocorticoids.

The antagonism of the histamine-induced protein efflux produced by arterial hemorrhage or pharmacologically induced systemic hypotension is attributable to an endogenous release of one or more vasoactive agents. Since the combined histamine-catecholamine infusion sometimes produces changes in mean aortic pressure different from those produced by histamine alone, it is possible that some of the observed effects could be attributable to an endogenous release of vasoactive substances. Norepinephrine infused with histamine usually failed to change or increased mean aortic pressure; hence, no reflex release of catecholamines would be predicted. Isoproterenol infused with histamine usually caused decreases in mean aortic pressure similar in magnitude to that produced by intra-arterial infusions of histamine alone; hence, any stimulus for an increased sympathoadrenal discharge would be similar under both conditions. Despite a similar stimulus for an increased sympathoadrenal discharge, only the infusion of histamine alone produced a marked increase in protein efflux. This agrees with the findings of Rippe and Grega (1978). These investigators demonstrated that, in the isolated, artificially perfused hindquarter vascular bed, isoproterenol largely prevents the hista-
mine-induced increase in protein efflux under conditions in which recirculation of flow is prevented.

References


The interrelationship among histamine, various vasoactive substances, and macromolecular permeability in the canine forelimb.

G J Grega, J J Maciejko, R M Raymond and D P Sak