Local Aortic Histamine Metabolism and Albumin Accumulation

Differences Between Blue and White Areas

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Relationships between histamine metabolism, histamine content, and albumin accumulation were examined in Evans blue dye stained areas (blue) and unstained (white) areas of normal canine aortas. Results indicated that, while no differences existed in histamine methyltransferase-mediated catabolism, both histidine decarboxylase-mediated histamine synthesis and the histamine content of blue regions were significantly greater ($p < 0.005$) than in contiguous white areas. Blue areas also showed significantly higher fluorescein-labeled albumin accumulation than white areas. By multiple regression analysis, a significant relationship ($r = 0.81$) was obtained between local aortic albumin accumulation and combined influences of local histidine decarboxylase activity and histamine content. The best predictor in this case was the local histidine decarboxylase activity. These data indicate that blue areas, believed to represent areas of spontaneous hemodynamic-induced vascular injury, have a larger nascent histamine pool than do contiguous white areas and that the distribution of histamine and histamine synthesis in the aorta is highly variable depending on the region examined. The data also suggest that local aortic histamine synthesis in blue areas may play a significant role in mediation of the increased albumin accumulation observed in these regions.


Endothelial injury has long been implicated as occupying a key role in the initiation of atherosclerosis. The current interest in endothelial injury has resulted from the recognition of morphologic similarities between the early stages of atherosclerosis and lesions observed in arteries subjected to a variety of experimental injuries. These similarities include smooth muscle proliferation, accelerated de novo connective tissue synthesis, and intra- and extracellular lipid deposition. Intimal proliferative lesions are believed to represent responses of the vascular wall to plasmatic and cell-derived substances entering in increased amounts as a result of increased endothelial permeability or localized endothelial denudation. Studies by Ross and coworkers, in which they identified blood-borne factors having mitogenic properties, have indirectly emphasized the importance of determining factors influencing local endothelial permeability. The protein-binding dye, Evans blue (EBD, T-1884), has routinely been used as a qualitative indicator of differences in local arterial endothelial permeability. It is generally accepted that EBD’s staining intensity is proportional to the degree of endothelial injury, although attempts to quantify EBD uptake are compromised by some dissociation of the dye-albumin complex within the vessel wall. Areas of localized dye uptake (blue areas), in addition to having higher endothelial turnover rates, exhibit increased accumulation of $^{131}$I-albumin, $^{131}$I-fibrinogen, and $^3$H-cholesterol. On the basis of numerous histological and ultrastructural studies of these
areas, sites of EBD uptake are believed to represent localized regions of spontaneous vascular injury created by chronic exposure to locally elevated hemodynamic stresses. Furthermore, blue areas are sites of lipid deposition in experimentally induced atherosclerosis.

Although it is generally agreed that blue areas show increased accumulation of a variety of macromolecular tracers, no information is currently available as to metabolic processes operating in blue areas that may be involved in mediation of these injury-induced increases. Hollis and coworkers have examined the possibility that atherosclerosis involves, at least in part, a mechanism similar to that of the delayed-prolonged inflammatory response of the microcirculation that is mediated to some extent by nonmast cell de novo histamine synthesis. A consistent finding has been that aortic histamine synthesis, mediated by histidine decarboxylase (HD), is increased in every case where an animal has been subjected to a known atherosclerotic risk factor. Under normal conditions, endothelial cells are the principal source of this elevated HD. From these studies the concept has emerged that injury-induced activation of the HD system may constitute one control mechanism in the regulation of endothelial permeability. In none of these studies, however, have possible relationships between histamine content and rates of histamine synthesis and catabolism been examined. Each of the above studies involved artificially induced vascular injury; we raise the question as to whether differences in histamine metabolite existence in normal aortas between local areas with spontaneous variations in permeability. The principal objectives of the present investigation were to use the EBD model to visualize areas of spontaneous injury, to quantify albumin accumulation in such areas, and to examine relationships between local albumin accumulation and local rates of histamine synthesis, histamine catabolism mediated by histamine methyltransferase (HMT), and histamine content.

Methods

Eight adult beagle-type mongrel dogs (Quaker Farm Kennels, Quakertown, Pennsylvania) weighing approximately 15 kg were used in this investigation. Three hours before sacrifice, the animals were injected intravenously with a 0.5% solution of Evans blue dye (EBD, T-1884, Fisher Scientific Company) in a sterile saline solution at a dosage of 3 ml/kg. In addition, the animals were given intravenous injections of 50 mg/kg fluorescein isothiocyanate conjugated to bovine serum albumin (FITCBSA, Fraction V, Sigma Chemical Company, St. Louis, Missouri) at time intervals of either 1 or 3 hours before sacrifice. Following FITCBSA injection, blood samples were obtained at 5, 30, and 60 minutes and at 60-minute intervals thereafter. These blood samples were allowed to clot and were centrifuged (10,000 g, 20 minutes). The serum obtained was frozen for subsequent serum FITCBSA determinations. Animals were lightly anesthetized by intravenous administration of 280 mg of Suritol (Parke-Davis) supplemented with 0.54 mg of atracrine sulfate. They were killed by air embolism. The aorta was perfused under low pressure with cold 0.05 M sodium phosphate buffer (PBS, pH 7.9), and the intact aorta from the valve ring to the diaphragm was removed and immersed in cold PBS (0.05 M, pH 7.9) on ice. The chilled aorta was stripped of periadventitial fat and adhering adventitia, opened longitudinally along the lesser curvature, and placed in fresh PBS on ice.

Areas of EBD accumulation (blue) were mapped on aortic diagrams. Aortas were quickly photographed, and tissue samples were taken from blue and immediately adjacent white areas using dermal punches of known diameters ranging from 3 to 8 mm. An intima-media preparation was then made from each plug using procedures described by Wolinsky and Daly involving dissection of adventitia from the intima media in the cold. plugs were identified with respect to aortic location, weighed, and homogenized in 10 to 40 volumes of PBS (0.01 M, pH 7.9) containing 0.1% w/v Triton X-100 using a ground-glass pestle. Approximately 1 ml of the homogenate was prepared for assays of HD activity, using Sephadex gel filtration as described by Levine and Watts. The remaining homogenate was centrifuged in a Sorvall centrifuge (4°C, 20 minutes, 20,000 g) for subsequent analyses of tissue FITCBSA, histamine, and protein contents, and HMT activity.

FITCBSA was used for quantification of protein accumulation as described by Katora and Hollis. Conjugation of BSA to FIT was achieved by modification of the procedure of Nairn. A 0.3 ml aliquot of the aortic supernatant (0.005%) or serum sample (0.0005%) was transferred to a microcuvette and the relative fluorescence measured on an MPF-43A Perkin-Elmer fluorescence spectrophotometer using wavelengths of 495 nm excitation and 523 nm emission. Fluorescence readings were compared to those obtained from a standard curve, and the amount of FITCBSA per sample determined and corrected for dilution. To assure that FITCBSA extraction from the aortic sample was complete, additional fluorescence readings were performed on samples of pellet subjected to NaOH digestion. Essentially no fluorescence (less than 0.05% relative to the supernatant fraction) was ever noted in the pellet fraction. In addition, precipitation of aortic homogenates with 10% (w/v) trichloroacetic acid removed more than 99% of
FITC fluorescence, indicating that free FITC in tissue was minimal. FITCBSA accumulation was expressed as a normalized mass transfer rate, \( J / \Delta C \). \( J \) equals \( C_s / W / \Delta t \), where \( C_s \) is the tissue concentration (ng FITCBSA/g aorta) of label, \( W \) is the weight (g) of aortic wall sample, \( A \) is the sample luminal surface area (cm\(^2\)), and \( t \) is the time of exposure to label. \( \Delta C \) is the concentration gradient (ng FITCBSA/ml plasma) of labeled albumin between tissue and plasma. Since \( C_s / C \) plasma was on the order of \( 10^{-3} \), \( \Delta C \) was closely approximated by \( C \) plasma.

Both the tissue histamine content and HD activity were determined by modification of the double isotopic microassay of Taylor and Snyder. For HD determination, initially a 25 \( \mu \)l tissue sample was diluted to 45 \( \mu \)l with 0.05 M PBS containing 0.25 mM pyridoxal phosphate at pH 6.2. Then 5 \( \mu \)l of 0.1 M L-histidine were added, and the tissue sample was incubated for 90 minutes (37°C). Blanks and standards were prepared with D-histidine. After stopping the reaction by boiling in a water bath for 10 minutes, the pH was adjusted to 7.9 with 0.1 M NaOH, and 20 \( \mu \)l samples were removed for analysis of the histamine produced. This second assay utilized imidazole-N-methyltransferase (HMT, E.C. 2.1.1.8) prepared from guinea pig brain, which methylates histamine to form 1-methyl \( \beta \)-aminoethyl imidazole (methylhistamine). In this procedure, 10 \( \mu \)l of 0.25 mM pyridoxal phosphate at pH 6.2. Then 5 \( \mu \)l of 0.1 M L-histidine were added, and the tissue sample was incubated for 90 minutes (37°C). Blanks and standards were prepared with D-histidine. After stopping the reaction by boiling in a water bath for 10 minutes, the pH was adjusted to 7.9 with 0.1 M NaOH, and 20 \( \mu \)l samples were removed for analysis of the histamine produced. This second assay utilized imidazole-N-methyltransferase (HMT, E.C. 2.1.1.8) prepared from guinea pig brain, which methylates histamine to form 1-methyl \( \beta \)-aminoethyl imidazole (methylhistamine). In this procedure, 10 \( \mu \)l of HMT were added to the sample containing histamine and incubated for 1 hour at 37°C. The HMT solution contained 1 part \( ^{14} \)C-S-adenosylmethionine (SAMe, 5 \( \mu \)Ci/ml, specific activity 58.4 mCi/mmole), 1 part \( ^{3} \)H-histidine (0.64 \( \mu \)Ci/ml, 6.1 \( \mu \)Ci/mmole), and 2 parts of HMT enzyme preparation. The resulting \( ^{14} \)C-methylhistamine and \( ^{3} \)H-\( ^{14} \)C-methylhistamine were recovered by extraction from an alkaline, salt-saturated solution into chloroform. Histamine content per sample was then determined through the ratio of \( ^{14} \)C: \( ^{3} \)H, following construction of standard curves from internal standards containing known amounts of histamine.

The following two modifications of the original procedure were made: 1) pyridoxal phosphate was added to the incubation medium with the buffer instead of the substrate to allow time for cofactor reaction with the enzyme before starting the reaction by addition of L-histidine; 2) HD assays were performed on tissue samples subjected to gel filtration (Sephadex G-25) instead of whole tissue homogenates. Samples were counted in Aquasol (Nuclear Associates, Westbury, New York) using a Tri-Carb scintillation spectrometer (Packard Instrument Company). Results of HD determinations were expressed in terms of nmole histamine formed \( \times \) hr \(^{-1} \) \times \) g \(^{-1} \) aorta, while histamine content was expressed as nmole histamine \( \times \) g \(^{-1} \) aorta. All samples were run in quadruplicate.

The HMT activity was measured by modification of the method of Taylor and Snyder. This assay measures the formation of \( ^{14} \)C-methylhistamine, with \( ^{14} \)C-SAME (58.4 mCi/mmole) serving as the methyl donor. Here, 15 \( \mu \)l of a freshly prepared solution containing 0.25 mM histidine, 5 \( \mu \)l of 8 mM SAMe, 100 \( \mu \)l of 0.1 mM \( ^{14} \)C-SAME, and 600 \( \mu \)l of 0.05 M PBS (pH 7.4) were added to 10 \( \mu \)l of tissue supernatant. This mixture was incubated at 37°C for 15 minutes and then placed in an ice bath. The reaction was stopped with addition of 8 \( \mu \)l of 1 M NaOH. The methylhistamine formed was then extracted from an alkaline, salt-saturated solution into chloroform. Again, samples were counted in Aquasol, and HMT activity was expressed as nmole methylhistamine formed \( \times \) hr \(^{-1} \) \times \) g \(^{-1} \) aorta. Blanks were prepared with boiled tissue supernatants. All samples were run in triplicate.

Statistical Analyses

Statistical analyses of data from blue and adjacent white aortic regions within a given dog for each specific parameter evaluated were performed utilizing the Student’s t test for paired data. Comparison of individual values between animals was achieved through use of the Behrens-Fischer t test, which does not assume equal variances between groups. Regression analyses, both linear and multiple, were done utilizing analysis of variance and the method of least squares.

Bluing Patterns

In all animals, bluing was observed in the ascending aorta and outer aortic arch. Additionally, in three animals there were occasional small patches of dye uptake just distal to the ductus scar; another three animals showed similar staining in the region of the sinus of Valsalva. Within the ascending aorta and outer aortic arch, three basic staining patterns were observed. In some animals the staining was homogeneous; tissue plugs taken from these aortas will hereafter be referred to as plugs OA. In other animals, staining consisted of blue patches in either or both the dorsal portion of the outer arch (DOA) and ventral portion of the outer arch (VOA). Figure 1 is a composite diagram of the staining patterns observed, together with area notations. Figure 2 shows the bluing pattern of a representative aorta (Dog 5). There was intense bluing in the ventral portion of the VOA, with additional blue patches present in both the sinus of Valsalva (SV) and area of the ductus scar (DS).
**Figure 1.** Composite of Evans blue dye (EBD) staining patterns in vivo in the normal dog thoracic aorta. Aortas were opened longitudinally along the lesser curvature. BC = brachiocephalic orifice; LS = left subclavian orifice; IC = intercostal orifices; OA = outer arch; DOA = dorsal section of outer arch; VOA = ventral section of outer arch. EBD injected 3 hours prior to sacrifice.

**Figure 2.** Representative photograph demonstrating pattern of luminal Evans blue dye (EBD) uptake in the normal dog. This aorta is from Dog 5, with the aorta being opened along the lesser curvature. Blue areas appear gray.

**Albumin Accumulation**

The aortic accumulation of FITCBSA at 1 and 3 hours after injection for plugs from specific blue and adjacent white regions is presented in Table 1, together with the corresponding means and standard errors. While the number of plugs examined in the SV and DS regions that showed bluing was not large enough for meaningful statistical analyses, it is immediately apparent that, in the aortic arch region, FITCBSA accumulation of blue areas was significantly greater (p < 0.01, paired t test) than in contiguous white regions. Within these blue areas, 1-hour values were significantly higher than the 3-hour values, while the corresponding 1- and 3-hour values for white areas were identical. While statistical analyses were not possible, it appears that values in the ductus scar region were somewhat lower than those of either the aortic arch or sinus of Valsalva regions.

**Table 1. Albumin Accumulation of Blue and Adjacent White Regions of the Normal Dog Aorta**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Blue area site*</th>
<th>FITCBSA postinject time (hrs)†</th>
<th>Albumin accumulation (×10⁻⁷ cm sec⁻¹)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>VOA</td>
<td>1</td>
<td>Blue 5.88 Contiguous white 5.29 ± 0.24</td>
</tr>
<tr>
<td>3</td>
<td>VOA</td>
<td>1</td>
<td>Blue 4.66 Contiguous white 5.29 ± 0.24</td>
</tr>
<tr>
<td>4</td>
<td>DOA</td>
<td>1</td>
<td>Blue 5.09 Contiguous white 5.29 ± 0.24</td>
</tr>
<tr>
<td>5</td>
<td>OA</td>
<td>1</td>
<td>Blue 4.97 Contiguous white 5.29 ± 0.24</td>
</tr>
<tr>
<td>6</td>
<td>VOA</td>
<td>1</td>
<td>Blue 5.83 Contiguous white 5.29 ± 0.24</td>
</tr>
<tr>
<td>7</td>
<td>OA</td>
<td>1</td>
<td>Blue 4.97 Contiguous white 5.29 ± 0.24</td>
</tr>
<tr>
<td>8</td>
<td>VOA</td>
<td>1</td>
<td>Blue 4.31 Contiguous white 5.29 ± 0.24</td>
</tr>
<tr>
<td>9</td>
<td>DOA</td>
<td>1</td>
<td>Blue 3.84 Contiguous white 5.29 ± 0.24</td>
</tr>
</tbody>
</table>

*OA = outer arch; VOA = ventral portion of the outer arch; DOA = dorsal portion of the outer arch; SV = sinus of Valsalva; DS = ductus scar. See figure 1 for diagram of aortic location.
†Fluorescein isothiocyanate conjugated to bovine serum albumin.
‡Normalized mass transfer.

$\text{ng FITCBSA}_{\text{tissue}} \times \text{cm}^2 \times \text{surface area} \times \text{sec}^{-1} \div \text{ng FITCBSA}_{\text{plasma}} \times \text{cm}^3 \times \text{plasma}$

§Blue region significantly greater than contiguous white region (p < 0.01, paired t test).
||Greater than 2 standard deviations from the mean. These data have been eliminated in all statistical analyses.
Histamine Metabolism

As indicated in figure 3, the double isotopic assay used for measurement of HD activity and tissue histamine content was linear between histamine concentrations of 0.1 and 2.0 ng per sample. Counting efficiency for 14C was 48% and for 3H was 18%.

Histamine content (nmole X g⁻¹), HD activity (nmole X hr⁻¹ X g⁻¹), and HMT activity (nmole X hr⁻¹ X g⁻¹) for these blue and adjacent white tissue plugs are presented in table 2. In the ascending aorta and aortic arch, both histamine content and HD activity of blue areas were significantly higher than similar values for adjacent white areas. A similar trend was seen for the sinus of Valsalva and ductus scar, although a meaningful statistical analysis of group differences in these cases is not possible because of the small sample number. No difference is evident in HMT activity between these same blue and white areas.

Regression Analyses

A series of regression analyses were performed on potential relationships between the various parameters evaluated. A correlation coefficient (r = 0.58, p < 0.05) existed between the local tissue histamine content and 1-hour albumin accumulation. As shown in figure 4, a significant relationship existed between the local tissue HD activity and the 1-hour albumin accumulation (r = 0.78, p < 0.005). A multiple regression analysis of 1-hour albumin accumulation to HD activity and tissue histamine content yielded the

Table 2. Histamine Content, Synthesis (HD), and Catabolism (HMT) In Blue and Adjacent White Regions of the Normal Dog Aorta

<table>
<thead>
<tr>
<th>Dog</th>
<th>Blue area site*</th>
<th>Histamine content (nmole X g⁻¹)</th>
<th>Histidine decarboxylase (HD) activity (nmole X hr⁻¹ X g⁻¹)</th>
<th>Histamine methyltransferase (HMT) activity (nmole X hr⁻¹ X g⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Blue</td>
<td>White</td>
<td>Blue</td>
</tr>
<tr>
<td>1</td>
<td>VOA</td>
<td>44.9±</td>
<td>27.4</td>
<td>27.9†</td>
</tr>
<tr>
<td>2</td>
<td>VOA</td>
<td>50.8†</td>
<td>36.6</td>
<td>31.1†</td>
</tr>
<tr>
<td>3</td>
<td>VOA</td>
<td>25.6†</td>
<td>0.0†</td>
<td>16.6†</td>
</tr>
<tr>
<td>3</td>
<td>DOA</td>
<td>38.2†</td>
<td>0.0†</td>
<td>13.7†</td>
</tr>
<tr>
<td>4</td>
<td>OA</td>
<td>50.1†</td>
<td>37.9</td>
<td>10.9†</td>
</tr>
<tr>
<td>5</td>
<td>VOA</td>
<td>82.7†</td>
<td>55.5</td>
<td>22.4†</td>
</tr>
<tr>
<td>6</td>
<td>OA</td>
<td>48.6†</td>
<td>18.4</td>
<td>10.2†</td>
</tr>
<tr>
<td>7</td>
<td>OA</td>
<td>81.5†</td>
<td>40.2</td>
<td>20.8†</td>
</tr>
<tr>
<td>8</td>
<td>VOA</td>
<td>62.1†</td>
<td>41.2</td>
<td>35.1†</td>
</tr>
<tr>
<td>8</td>
<td>DOA</td>
<td>25.7†</td>
<td>15.4</td>
<td>0.0†</td>
</tr>
<tr>
<td>mean ± SE</td>
<td></td>
<td>51.0±6.3</td>
<td>27.3±5.8</td>
<td>18.4±3.5</td>
</tr>
<tr>
<td>2</td>
<td>SV</td>
<td>40.7</td>
<td>36.2</td>
<td>19.5</td>
</tr>
<tr>
<td>5</td>
<td>SV</td>
<td>88.5</td>
<td>40.2</td>
<td>12.5</td>
</tr>
<tr>
<td>7</td>
<td>SV</td>
<td>39.5</td>
<td>55.5</td>
<td>21.3</td>
</tr>
<tr>
<td>mean ± SE</td>
<td></td>
<td>56.2±6.1</td>
<td>43.9±5.9</td>
<td>17.8±2.7</td>
</tr>
<tr>
<td>4</td>
<td>DS</td>
<td>48.7</td>
<td>28.7</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>DS</td>
<td>24.7</td>
<td>14.4</td>
<td>13.4</td>
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<tr>
<td>8</td>
<td>DS</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>mean ± SE</td>
<td></td>
<td>36.7±12.0</td>
<td>21.5±7.2</td>
<td>—</td>
</tr>
</tbody>
</table>

*See figure 1 for aortic location.
†Significantly greater than white region (p < 0.005, paired t test).
‡Below limits of assay sensitivity (histamine, 2 nmoles X g⁻¹; HD, 5 nmoles X hr⁻¹ X g⁻¹; HMT, 5 nmoles X hr⁻¹ X g⁻¹).
equation \( y = (0.28 x_1 + 0.06 x_2 + 3.49) \times 10^{-3} \), where \( x_1 \) is the HD activity and \( x_2 \) the tissue histamine content. The correlation coefficient \( (r) \) was 0.81; the \( T \) ratio of \( x_1 \) was 3.67 (\( p < 0.01 \)) while that of \( x_2 \) was 1.91 (\( p < 0.05 \)), thus indicating that HD activity is a stronger predictor of albumin accumulation than is the local vessel histamine content. A significant relationship was also noted between the local tissue histamine content and local HD activity \( (r = 0.51, p < 0.05) \).

**Discussion**

**Albumin Accumulation**

In the present study, the EBD staining patterns observed in normal dogs are in agreement with those previously described by others for a variety of mammalian species.\textsuperscript{13, 16, 23, 24} Likewise, results of the present investigation confirm earlier studies\textsuperscript{13, 16} showing an increase in labeled albumin accumulation in blue regions relative to adjacent white areas. This increased accumulation is consistent with, but is not conclusive evidence for, an increase in endothelial permeability due to a number of limitations in the method. It cannot be ascertained from the present study whether the increase in albumin accumulation is due to increased permeability, decreased clearance, or an increase in the albumin distribution space in blue areas relative to white areas. However, regardless of the mechanism, results do provide evidence of functional injury in the blue areas, findings that are consistent with morphological observations reported by others.\textsuperscript{19-21}

**Histamine Metabolism**

A major objective of this study was to characterize the local distribution of histamine within the normal aorta and to examine histamine distribution in relation to local aortic histamine metabolism. The significant findings in this respect are that EBD stained areas have up to twice the intravascular histamine content of adjacent white areas, and that these same areas have markedly elevated rates of histamine synthesis. Indeed, in all but one case, HD activity of white aortic plugs was below the limits of assay sensitivity (5 nM/hr/g). Histamine catabolism mediated by HMT, which is the principal histamine catabolic enzyme in the dog,\textsuperscript{41, 42} is constant between aortic regions. These represent new findings, since previous measurements of vascular histamine have been based on that within homogenates of an entire vessel.\textsuperscript{43-45}

With respect to the source of vascular histamine, it is important to note that statistical analyses indicate that a small yet significant portion of the variance in aortic histamine content can be accounted for by differences in vascular histamine synthesis. Furthermore, based on the relative magnitudes of histamine content, HD activity, HMT activity, and previous reports of rates of histamine turnover,\textsuperscript{46} it is evident that a major component of the measured histamine must be from some source other than arterial HD. One source might be plasma, since Foldes et al.\textsuperscript{45} have shown that aortic segments incubated with labeled histamine in vitro accumulate histamine against a concentration gradient. Other sources could be platelets or leukocytes, both of which are found in increased numbers in blue areas, as well as mast cells.\textsuperscript{19, 20, 47}

Kahlson and Rosengren\textsuperscript{48} and Levine and Noll\textsuperscript{49} have described three metabolic pools of histamine present in mammalian tissue. Two of these have slow turnover rates and consist of histamine bound in an inactive form. While one of these is associated with mast cells and can be depleted by compound 48/80, the other is not yet fully characterized and its depletion has not been achieved. However, both are associated with low HD activities. The third pool, nascent or inducible histamine, consists of unbound and active histamine. Its magnitude is determined by the balance between synthesis and catabolism, and it is readily depleted by inhibition of HD. It is this pool to which physiological functions such as regulation of gastric acid secretion and intrinsic microcirculatory flow have been ascribed.\textsuperscript{26, 50} In the rabbit, Foldes et al.\textsuperscript{45} have shown that 37% of arterial histamine is present in this pool. Based on evaluation of the statistical data, our results indicate that the magnitude of this pool is highly variable, depending on the aortic region involved. In white areas, it presumably constitutes a mini-
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In the number of active vesicles and that such activation occurs by increased synthesis of some unidentified factor. We propose that HD-mediated histamine synthesis might be one such factor involved in this process, since Renkin et al. have offered mathematical evidence that histamine stimulates pinocytotic transport of dextran in dog forepaw microcirculation. With respect to junctional transport, Majno et al. have described histamine-induced microcirculatory changes in endothelial morphology (i.e., "endothelial contraction"). If this process is present in large arteries, it would necessarily alter interendothelial junctional transport. In addition, histamine may cause local tissue edema, which has been observed in morphologic studies of blue areas, and thereby increase albumin accumulation by increasing the arterial albumin distribution space.

In summary, data from this study have demonstrated that local areas of increased albumin accumulation in the normal dog aorta have a significantly higher HD activity and histamine content than adjacent white areas, while histamine catabolism mediated by HMT is constant. Data suggest that other sources of vascular histamine are also present, but that in blue areas a significant component of the measured histamine is that of the nascent, physiologically active histamine pool. Based on present evidence of functional injury in blue areas, as well as on previous descriptions of morphologic alterations in these areas, sites of EBD uptake are believed to represent areas of focal vessel injury created by chronic exposure to locally elevated hemodynamic stresses. Our results suggest that altered histamine metabolism may be one metabolic manifestation of spontaneous vascular injury, with increased histamine synthesis occupying an important role in the mediation of injury-induced local albumin accumulation.

References

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Interrelationships

As noted above, sites of EBD uptake within normal arteries are believed to represent localized regions of spontaneous injury created by chronic exposure to locally elevated hemodynamic stresses. Data from this study show that these blue areas have high rates of histamine synthesis and related high intravascular histamine contents with respect to adjacent white aortic regions. These data suggest that the aortic HD system in normal vessels is sensitive to local flow conditions and that, under conditions of disturbed flow, one local reaction of the vessel wall is increased histamine synthesis with a resultant elevation in the local nascent, physiologically active histamine pool. This is consistent with previous findings that vascular histamine synthesis is increased with artificially elevated shear stress exposure.

From this study it is also apparent that the blue regions of the aorta show, in addition to high rates of histamine synthesis and a high histamine content, significantly greater albumin accumulation than adjacent white regions. This observation suggests a possible causal role of vascular histamine in influencing local aortic uptake of albumin under normal conditions. This concept is supported by data obtained from regression analyses in this study and also by previous studies in which aortic albumin permeability was reduced under dietary-induced hypercholesterolemia by partial inhibition of histamine synthesis. Together, these data suggest that one metabolic manifestation of spontaneous injury is increased endogenous histamine synthesis, with the resulting nascent histamine producing locally increased wall accumulation of circulating macromolecules such as albumin.

Assuming that the HD system is involved in mediation of albumin accumulation, especially in blue areas of the aorta, we must address the question of the mechanism involved. In an examination of endocytotic vesicles in endothelial cells in different aortic regions, Schwartz et al. observed no quantitative differences in vesicle numbers between blue and white areas but did show that the number of ferritin-loaded vesicles in blue areas is significantly higher than in white aortic regions. They proposed that one manifestation of vascular injury in these regions is an increase
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Local aortic histamine metabolism and albumin accumulation. Differences between blue and white areas.

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