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Variations in Rabbit Aortic Endothelial and Medial Histamine Synthesis in Pre- and Early Experimental Atherosclerosis[1] (39808)

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Introduction. A working premise of our laboratory has been that atherogenesis may involve, at least in part, a mechanism similar to that of the delayed-prolonged inflammatory response. In this regard, our focus of investigation has been nonmast cell, *de novo* histamine of the arterial wall (1), synthesized by mammalian L-histidine apodecarboxylase (E.C. 4.1.1.22), hereafter referred to as histidine decarboxylase. As measured through determination of the *in vitro* histamine-forming capacity (HFC), we have reported marked increases of aortic histidine decarboxylase activity following exposure of aortas to various atherogenic stresses, such as those associated with elevated shear stress (2, 3), in both neurogenic and mechanical hypertension (4, 5), and in rabbits showing moderate hypercholesteremia (6). With respect to the latter study, in preatherosclerotic rabbits we have recently demonstrated that the HFC of aortic endothelial cells is elevated while that of subjacent medial smooth muscle is unchanged (7). The present study was designed to examine histidine decarboxylase activity in both endothelial and medial components of rabbit aortas which show both hypercholesteremia and no evidence of aortic lipidosis, as well as in aortas showing evidence of early atherosclerotic lesions. This has been done in order to determine whether the change in enzyme activity is of a transient or a more permanent nature. If transient, then the system could be one which might, through its product histamine, be involved in initial increases in endothelial permeability, and could well represent at least one mechanism which can couple altered vascular states to subsequent increases in arterial wall uptake of blood-borne macromolecules.

Materials and methods. Male, Dutch-belted rabbits, 6-8 weeks old, were randomly divided into treatment groups which received either Purina Rabbit Chow or Purina Rabbit Chow supplemented with 0.5% cholesterol (w/v) for either a 2- or 4-week period. Food and water were administered ad libitum. At the end of the dietary period, and immediately prior to sacrifice, 10 ml of blood were obtained from each animal for determination of total serum cholesterol (TSC) levels, as described by Levine and Zak (8) and adapted for use with the Technicon Auto-Analyzer.

Following sacrifice by stunning, the complete thoracic aorta was removed. A small segment (5 mm) of the ascending arch was excised and immediately quick-frozen for subsequent histological examination. The remaining aortic tissue was immersed in cold phosphate-buffered saline (PBS, pH 7.2, 4°). Endothelial cells were isolated by modification of the method of Hollis and Rosen (9) as previously described in detail for rabbit tissue (7). Briefly, this method entails washing the inverted lumenal surface with a freshly prepared 0.1% trypsin solution in PBS for a series of 15-min periods. The endothelial cells thus removed are recovered by low-speed centrifugation at 4°, rinsed in cold PBS (4°), and pooled with the cells obtained from another rabbit of the same dietary group. Such pooling is necessary in order to obtain quantities of cells sufficient for subsequent biochemical distributions.

The pooled cells were stored frozen in 3 ml of PBS. A 5% (w/v) homogenate was prepared in PBS from the remaining trypsin-washed aortic tissue. Enzyme-containing supernatants of both endothelial and medial tissue preparations were obtained by high-speed centrifugation (10,000 rpm) for 20 min at 4°.

HD activity of the endothelial and medial tissue samples was determined by modifica-

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tion on the $^{14}CO_2$ trapping method of Levine and Watts (10) as has been previously described (2, 7, 9). The incubation mixture contained 1.85 ml of enzyme-containing supernatant, 0.05 ml of 1 m*M* pyridoxal-5-phosphate, and 0.1 ml of L-[^{14}C]histidine (carboxyl labeled, 1 μCi/ml). Blanks contained 10% trichloroacetic acid (TCA) instead of supernatant. Radioactivity was counted via standard liquid scintillation counting procedures. Results were corrected for background and expressed as a tissue sample's histamine-forming capacity, *i.e.*, disintegrations per minute per milligram of supernatant protein (dpm/mg). Protein content of the supernatant was determined by the method of Lowry *et al.* (11) using Folin reagent.

The method of Warren (12) for assessment of fibrinolytic activity was used as biochemical verification that the recovered cells were endothelium. Thin sections (8 μm) were cut from each frozen specimen for routine hematoxylin and eosin (H&E), and oil red-O staining as outlined in Humason (13), and were photomicrographed immediately after staining.

Results. All animals on the cholesterol dietary regimen exhibited significant ($P < 0.005$) hypercholesteremia with respect to pair-fed controls. Mean serum cholesterol (TSC) levels for the 2 and 4-week dietary groups were 324 and 551 mg, respectively, while control animals had a mean TSC of 61 mg.

H&E-stained cross sections of upper thoracic aortas showed no differences of wall integrity between the control and 2-week dietary groups, i.e., aortas from both groups showed a continuous lining of cells adhering to an intact internal elastic membrane overlying normally organized layers of medial smooth muscle and elastic lamellae. H&E-stained specimens from the 4-week animals were generally the same as those from the control and 2-week groups, with preparations from several animals exhibiting intimal disruptions characteristic of early lipemic lesions (14). Fresh-frozen cross sections subsequently stained with oil red-O lacked any discernible lipid deposition in the control and 2-week animals; in all cases, however, animals of the 4-week group exhibited early intimal and subintimal lipid deposition. Similar observations, together with photomicrographs, have been previously published (6).

HFC values for isolated thoracic endothelial cells are presented in Table I. Each value represents a single HFC determination of pooled endothelial cells from two thoracic aortas or rabbits of the same dietary group. The mean control, 2-week, and 4-week endothelial HFC values are 527 ± 60, 982 ± 108, and 480 ± 117 dpm/mg, respectively; the valve of the 2-week treatment group is significantly higher ($P < 0.01$) than that of either control or 4-week treatment groups. Table II shows the results of HFC determinations made on 5% aortic homogenates prepared from aortas previously subjected to lumenal-surface trypsin washing. The mean control medial HFC was 106 ± 10 dpm/mg, while the mean 2- and 4-week HFC values were 93 ± 3 and 82 ± 15 dpm/mg, respectively. These differences are not significant ($P > 0.05$).

These data thus show that in Dutch-belted rabbits, the mean thoracic aortic endothelial cell HFC is significantly increased after 2-weeks feeding of a 0.5% cholesterol diet; however, after 4 weeks of cholesterol feeding, the mean endothelial cell HFC is comparable to that of control animals. The medial HFC remains unchanged throughout the experimental period examined. All rabbits given cholesterol were hypercholesteremic, while histological examination of aor-

TABLE I. THORACIC AORTIC ENDOTHELIAL[a] HISTAMINE-FORMING CAPACITY (HFC)[b] OF RABBITS FED 0.5% CHOLESTEROL FOR 2 AND 4 WEEKS.

Replication number	Control	2 Weeks	4 Weeks
1	483	1366	350
2	386	1169	276
3	397	900	146
4	596	678	525
5	541	1058	943
6	786	723	640
Mean	527	982*	480
± SE	±60	±108	±117

[a] Each value represents a single HFC determination of pooled endothelial cells from two aortas of pair-fed rabbits.

[b] Disintegrations per minute per milligram of supernatant protein (dpm/mg of protein).

* Difference from mean control and 4-week values significant ($P < 0.01$, Student's t test).

TABLE II. THORACIC AORTIC MEDIAL[a] HISTAMINE-FORMING CAPACITY (HFC)[b] OF RABBITS FED 0.5% CHOLESTEROL FOR 2 AND 4 WEEKS.

Relication number	Control	2 Weeks	4 weeks
1	112	86	30
2	132	93	95
3	80	105	95
4	90	90	140
5	86	92	65
6	134	93	65
Mean	106	93*	82*
± SE	±10	±3	±15

[a] Five percent homogenate of previously trypsinized thoracic aorta. The endothelium was removed as a result of the trypsin treatment.

[b] Disintegrations per minute per milligram of supernatant protein (dpm/mg of protein).

* Difference from control not significant ($P > 0.05$, Student's t test).

tic tissues showed a lack of any lipemic lesion development prior to 2-weeks feeding of this cholesterol diet.

Discussion. As noted by Maier (15), the severity of atherosclerotic lesions in rabbits is dependent upon both duration and the level of hypercholesteremia. Gaman *et al.* (16, 17) and Adams *et al.* (18) have shown that Dutch-belted rabbits are more lesion resistant than New Zealand white rabbits, although both rabbit breeds show essentially similar hypercholesteremic responses following administration of cholesterol. This relative lesion resistance is a necessary component of the experimental design of the present investigation, since the intent has been to examine histidine decarboxylase activity in aortic wall components under a preatherosclerotic state as well as in an early stage of atherosclerosis. The histological appearance of aortas in each treatment group indicates that both states have been achieved, and photomicrographs supporting this conclusion have been published previously (6).

Becker and Murphy (19) have given conclusive evidence that aortic endothelium contains contractile protein antigenically similar to that of uterine smooth muscle. This offers a molecular basis for endothelial contraction orginally described by Majno and Palade (20) in rat cremaster microcirculation following topical application of histamine to the vascular bed. Employing electron microscopy, Constantinides and Robinson (21) have identified a similar pattern of endothelial contraction in aortas following exposure to pharmacological doses of various vasoactive amines and to a variety of injurious stimuli. A natural consequence of such contraction is an increase in the ratio of patent to closed interendothelial junctions as well as in the size of these patent junctions. Since interendothelial junctions constitute a functional small-pore system in the aorta (22), Constantinides and Robinson (21) and Robertson and Khairallah (23) have suggested that endothelial contraction can easily account for at least one component of increased endothelial transmural permeability, a principal event in the atherogenic process. However, the present study does not deal with ultrastructural changes of aortic endothelium. Thus, electron microscopic analyses would be required to provide direct evidence that the cholesterol regimen used in this study resulted in endothelial contraction, and that alterations in interendothelial junctional width are related to endothelial histamine formation.

DeForrest and Hollis (24) have recently examined the relationship between aortic steady-state albumin uptake and the aortic HFC in rabbits whose aortas were subjected *in situ* to pulsatile perfusion with deplateleted blood. They observed a correlation coefficient of 0.74 between these two parameters, suggesting that regional aortic histamine production is associated with regional aortic albumin permeability. Our laboratory has also shown that one characteristic of aortas in a preatherosclerotic state is an increase in aortic HFC (6). Since the histidine decarboxylase activity of aortic endothelium is essentially 15 times that of subjacent medial tissue (9), it is reasonable to assume that this endothelial enzyme system should contribute significantly to the total aortic HFC; this has been verified in a previous study involving the same experimental design but with administration of cholesterol for only 2 weeks (7), in which we reported that the increase in HFC was seen only in the endothelium. The findings of the present study indicate that this increase in endothelial HFC is transient, being present in preatherosclerotic aortas but not in those

aortas showing grossly visible evidence of atherosclerotic lesions; this transience is consistent with the adaptive nature of this enzyme system (25).

In light of the observed potential relationship between the aortic HFC and aortic steady-state albumin uptake (24), since the endothelium has at least a high *in vitro* capacity to form histamine (9), since histamine can increase endothelial permeability through promotion of endothelial contraction, at least in microcirculation (20), and since these cells show an increased *in vitro* capacity to form histamine in the preatherosclerotic state, the data of the present study give further credence to our premise that alterations in aortic histamine synthesis occupy a central role in the increased aortic permeability associated with atherogenesis. These data also offer an explanation for the antiatherogenic effects of histamine H_1 receptor antagonists which have recently been reported by Hollander *et al.* (26).

Summary. The histamine-forming capacity (HFC) of aortic endothelium and medial tissue has been examined in rabbits fed 0.5% cholesterol for 2- and 4-week periods, thereby producing both a preatherosclerotic and histologically identifiable early stage of atherosclerosis. With respect to the HFC of endothelium isolated from aortas of control animals, that of cells isolated from aortas following the 2-week feeding period is essentially double, while no difference exists between the HFC of aortic endothelium from control animals and that of the 4-week dietary group. The medial HFC is similar in all groups. These data thus suggest that one preatherosclerotic metabolic change is an increase in the capacity of endothelial cells to form histamine, and that such a process may occupy a role in increased aortic permeability, an essential component of the atherogenic process.

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