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Significance of Endothelial Cell Histidine Decarboxylase Activity

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Isolated endothelial cells have been shown to have a 15-fold greater histidine decarboxylase activity than adjacent intima-media homogenates. The significance of this is discussed in regard to microcirculatory regulation and atherogenesis. The newly formed histamine is thought to play a role in microcirculatory regulation by acting on "dilator receptors" at the capillary level. The role of histamine in atherogenesis is thought to be mediated through an increased arterial wall permeability. Specifically, the increased permeability is the result of increased endothelial cell pinocytotic activity and endothelial cell contraction. These contractions eventually cause distinct gaps in the endothelium at the inter-endothelial cell junctions. Data concerning histidine decarboxylase activity in hypertensive rat aortas is discussed in relation to measures of aortic permeability.

INDEX DESCRIPTORS: Histamine, Histidine Decarboxylase, Endothelial Cells, Hypertension, Microcirculatory Regulation.

Recent work has demonstrated that isolated endothelial cells obtained from bovine aorta showed a 15-fold greater histidine decarboxylase (HD) activity than did adjacent intima-media homogenates. HD is a specific L-histidine decarboxylase which decarboxylates the amino acid histidine to form the primary amine, histamine. The enzyme requires pyridoxal phosphate as a cofactor (Hakanson, 1967), and the activity of the enzyme is termed the histamine forming capacity (HFC) of the tissue (Kahlson and Rosengren, 1968). Hollis and Rosen (1972) found that isolated bovine endothelial cells had an HFC of 4371 ± 806 DPM/mg of protein, whereas adjacent intima-media homogenates had an HFC of 314 ± 46 DPM/mg of protein. This is a 15-fold difference and is significant because it supports Schayer's intrinsic microcirculatory homeostasis hypothesis (Schayer, 1962) and it strengthens data concerning the role of histamine in atherogenesis.

Microcirculatory Regulation

Schayer's hypothesis postulates a fairly simple interrelationship between glucocorticoids and induced histamine. Induced histamine is the newly synthesized histamine formed by the inducible HD enzyme system. The microcirculation is under continual influence of the newly synthesized histamine, which acts on so-called "dilator receptors" to keep tissue blood flow at a constant level. Further, the synthesis of histamine can be altered by many non-specific stresses, in an attempt to alter blood flow. For example, in thermal injury there is an increased HD, which is followed by a hyperemic response (Schayer and Ganley, 1959). Glucocorticoids serve to antagonize histamine by competing for these receptor sites. Normal microcirculatory homeostasis involves maintaining a balance between glucocorticoid levels and histamine synthesis. Schayer states that for histamine to be intrinsically active, the site of synthesis must be near or within the endothelial cells or microvascular smooth muscle cells. The previously cited data demonstrate that the site of synthesis is indeed within the endothelial cells and thus further support the concept that microcirculatory homeostasis is intrinsically regulated by induced histamine.

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Atherogenesis

The initial event in atherogenesis is thought to be increased arterial wall permeability. Hypertensive animals are more susceptible to atherosclerosis, and hypertension, as a non-specific stress, could serve as a stimulus for HD activation. For this reason, aortas from rats made hypertensive by aortic coarctation between the origins of the renal arteries and aortas from paired, sham operated control rats were compared in regard to HD activity and net permeability alterations as reflected by water and protein content.

MATERIALS AND METHODS

Male Holtzman rats weighing 300-500 mg were divided into two groups, experimental hypertensive rats and sham operated, normotensive control rats. Paired control (sham operated, normotensive) and hypertensive animals were used in each group for evaluation of the HFC, protein content and water content. All data were statistically analyzed according to the methods of Mendenhall, 1968.

Experimental hypertension was induced by the method of Rojo-Ortega and Genest (1968). This involves ligating the abdominal aorta between the origins of the renal arteries with the animal under light ether anesthesia.

At the end of the postoperative time period, each animal was weighed and sacrificed by cervical dislocation. The thoracic aorta, delimited by the left subclavian artery and the diaphragm, was excised, minced and homogenized with 20 volumes of 0.05 M sodium potassium phosphate buffer, pH 7.2. The temperature of the solution was maintained at 4°C. The resulting homogenate was placed in a plastic capped vial and stored at 0°C for one week. The homogenate was then thawed and the various procedures were performed.

Histidine decarboxylase activity was determined by modification of the method of Levine and Watts (1966). This involves quantifying the $^{14}CO_2$ liberated from ^{14}C-carboxyl-L-histidine following a two-hour incubation period in the presence of the enzyme aortic supernatant and cofactor. The enzyme was prepared by thawing of the aortic homogenate, followed by centrifugation at 10,000 rpm (20 min. 4°C) in a Sorvall superspeed centrifuge. The results were expressed as disintegrations per minute (DPM) per mg *supernatant* protein. All results were taken from duplicate analyses.

The protein content was determined by using the method of Lowry, *et al.* (1954). Results were expressed as mg protein per mg tissue wet weight.

Water content was determined by subtracting the aortic dry weight, obtained by incubating the tissue in a weighing vial at 100°C for 24 hours, from the previously measured wet weight. Results were expressed as mg water per mg dry weight.

TABLE 1. PERCENTAGE CHANGE IN RAT AORTIC HISTIDINE DECARBOXYLASE ACTIVITY (HFC), WATER CONTENT AND PROTEIN CONTENT THROUGH EIGHT DAYS OF HYPERTENSION.

Duration of Hypertension (Hours)	HFC	Protein Content	Water Content
6 hrs	+38%*	+83%*	+60%*
12 hrs	+59%	+65%*	+30%
24 hrs	+106%*	+93%*	+18%
48 hrs	+1.7%	.....	+107%*
96 hrs	−4.4%	+84%*	+12%
144 hrs	+3.4%	.....	+2%
196 hrs	+119%*	+141%*	+47%*

* $P \leq 0.05$

RESULTS

Table 1 summarizes the data collected in the study, giving the percentage change in rat aortic HFC, water content and protein content through eight days of hypertension.

Within six hours after induction of hypertension, there is a 38 percent increase in aortic HFC, which peaks at one day with a 106 percent increase. After a regression to essentially control levels, there is another area of increased activity after eight days. At this time the HFC shows a 119 percent increase as compared to paired, sham operated control values. The first area of increased activity appears to be a direct effect of the elevated blood pressure, because of its rapid onset. The second area may be mediated by some neural or hormonal mechanism, or possibly it is a response elicited by further non-specific hemodynamic or hydrostatic stress.

The aortic water content increases at six hours and then regresses until it is near control levels at day one. This initial increase at six hours is probably a result of the increased hydrostatic pressure driving aqueous substances into the wall. The exact mechanism of this infiltration is not known, but this water is immediately (from six hours to one day) extruded from the wall and returned to the circulation. The water content, with a one-day lag, then parallels the HFC data. Specifically, as the HFC increases, so does the water content. In relation to the second area of increased HFC, the water content directly parallels the HFC data.

There is an initial increase (83 percent) in aortic protein content within six hours after induction of hypertension, which slightly regresses at the 12-hour stage to 65 percent. This increase in protein is probably due to the increased hydrostatic pressure driving serum proteins into the wall. There is a return to higher levels at day one (93 percent), at which point it stays relatively constant until day eight, when it increases to 141 percent. The protein content directly follows the HFC data throughout the eight-day experimental period.

DISCUSSION

It appears from the data that the water content and protein content, and thus permeability, are greatly affected by the activity of HD. The protein content directly parallels the HFC and the increasing water content, after a lag of one day, parallels the HFC.

It is proposed that the increased HD activity, with a concomitant increased histamine synthesis, which is caused by the hypertension, is directly causing the increased permeability by causing increased endothelial pinocytosis and endothelial cell contraction. Histamine is known to be able to cause contraction of smooth muscle, a fact that is used as a bioassay for histamine, and endothelial cells are thought to be morphogenetically related to smooth muscle (Majno, *et al.*, 1969). Also, smooth muscle cell-like filaments have been observed within endothelial cells (Suzuki, *et al.*, 1971) and Becker and Murphy (1969) have shown that there is actomyosin within endothelial cells that is antigenically similar to uterine smooth muscle.

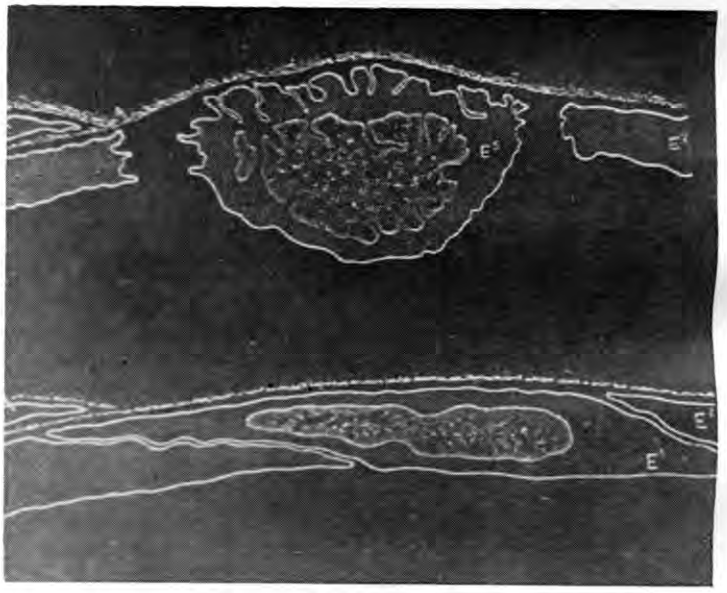

Figure 1. Endothelial cell contraction resulting in the formation of distinct gaps in the endothelial lining (courtesy of Majno, *et al.*, 1969).

Histamine injections have been shown to cause increased pinocytosis and to cause active endothelial cell contraction (Majno, *et al.*, 1969). It is obvious how increased pinocytosis would cause an increased influx of material into the wall.

When endothelial cells contract, they are observed to be lifted off adjoining endothelial cells, thus forming distinct gaps in the endothelial lining at the inter-endothelial cell junctions (Figure 1) (Majno, 1970). These types of gaps have been documented as the cause of the increased permeability

found in hypertension (Hunter, *et al.*, 1970). Therefore, it appears that induced histamine, via the enzyme histidine decarboxylase, is playing a significant role in this process.

A possible cause for the protein content to follow the HFC directly, and for the water content to have a one-day lag before it too follows the HD activation, is that there may be two different thresholds for contraction and stimulation of pinocytotic activity. Possibly the aqueous substances use one portal of entry and the protein and other macromolecules use the other portal of entry. Which portal of entry is used for each substance cannot be determined at this point.

SUMMARY

The initial event in atherogenesis is thought to be increased arterial wall permeability; histamine-induced endothelial cell contraction and increased endothelial cell pinocytotic activity have been implicated in the process. The increased aortic enzyme activities observed presumably reflect the activity of the endothelial cells. The demonstration that HD is localized within the endothelium helps substantiate the concept that histamine is playing a major role in atherogenesis, since the site of synthesis and the site of action are within the same cells.

Basic to Schayer's intrinsic microcirculatory regulation hypothesis is that the induced histamine that controls capillary dilation is formed within or near the endothelial cells. Once again, the demonstration that the site of synthesis (of the histamine) and the site of action are within the same cell greatly adds credibility to the hypothesis.

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