Histidine Decarboxylase Activity of Bovine Aortic Endothelium and Intima–Media[1] (36914)

THEODORE M. HOLLIS AND LEE A. ROSEN
(Introduced by A. Anthony)
Biology Department, 208 Life Sciences I, The Pennsylvania State University University Park, Pennsylvania 16802

Mammalian L-histidine apodecarboxylase (EC 4.1.1.22), hereafter referred to as histidine decarboxylase (HD), is present in most tissues (1). Activation of this system in the microcirculation produces the delayed-prolonged inflammatory response (2), and Schayer (3–5) has proposed that histamine synthesized by this system in the microcirculation constitutes a primary mechanism of intrinsic microcirculatory regulation. Basic to this premise is his proposal that the principal site of HD is microcirculatory endothelium, a premise which to date has not been experimentally verified.

The present study was undertaken to examine the activity of HD in both endothelium and in other components of the vascular wall in an attempt to test this basic assumption of Schayer's intrinsic microcirculatory control hypothesis.

Methods. Animals. All steers were raised on The Pennsylvania State University-owned farms, with sacrifice and tissue removal being performed in the university's Meat's Laboratory. Following sacrifice by stunning, the thoracic aorta of each animal was removed, opened longitudinally, and placed in cold phosphate-buffered saline containing 12 m*M* glucose (PBS).

Endothelial cell isolation. Endothelial cells were removed enzymatically through inverted luminal washing with an 0.25% trypsin solution. A 4 × 7 cm rectangular section of tissue was removed from the midthoracic aorta, following which an 0.5 mm thick intima–media preparation was prepared. Cyanoacrylate glue (Allen Tool Co., Philadelphia, PA), which produces limited cell necrosis and has been successfully used in various surgical procedures (6), was used to attach the exposed medial surface to a 12 × 75 mm glass tube. This tube was then inserted into a 22 × 90 mm glass tube containing 0.25% trypsin (Bacto-Difco 1:250, Difco Laboratories, Detroit, MI) in PBS and a magnetic stirring bar, and was brought to and maintained at 37° by means of a constant temperature water bath setting on a magnetic stirrer. Optimal trypsin washing time was determined by examining aliquots of solution at 5 min intervals through phase microscopy. The trypsin–PBS solution was changed at 15 min intervals using capillary pipets.

Removal of small thrombi and initial endothelial loosening required two, 10 min washings, with both washings being discarded. Three additional 15 min washings yielded the majority of free endothelial cells. Subsequent washings produced only very low numbers of cells or cell fragments. Cells in each of the three, 15 min washings were collected by first cooling the suspension to 4° to reduce trypsin activity, followed by centrifugation for 5 min at 1000*g*. The supernate was discarded and the cell pellet was resuspended in 1 ml of Krebs–Ringer phosphate solution containing 12 m*M* glucose (KRP). All 3 suspensions were pooled, centrifuged, washed, and finally resuspended in 1.5 ml KRP. The pellet was rapidly frozen and thawed to lyse cells and then centrifuged at 10,000*g* for 20 min (4°). The supernatant, containing the soluble enzyme (7), was stored at −20° for subsequent enzymatic studies. Storage never exceeded 2 wk.

Intima–media preparation. A 10.0% homogenate of midthoracic aortic intima–media

[1] This investigation was supported in part by the Lancaster County Chapter of the Pennsylvania Heart Association, PHA3505.

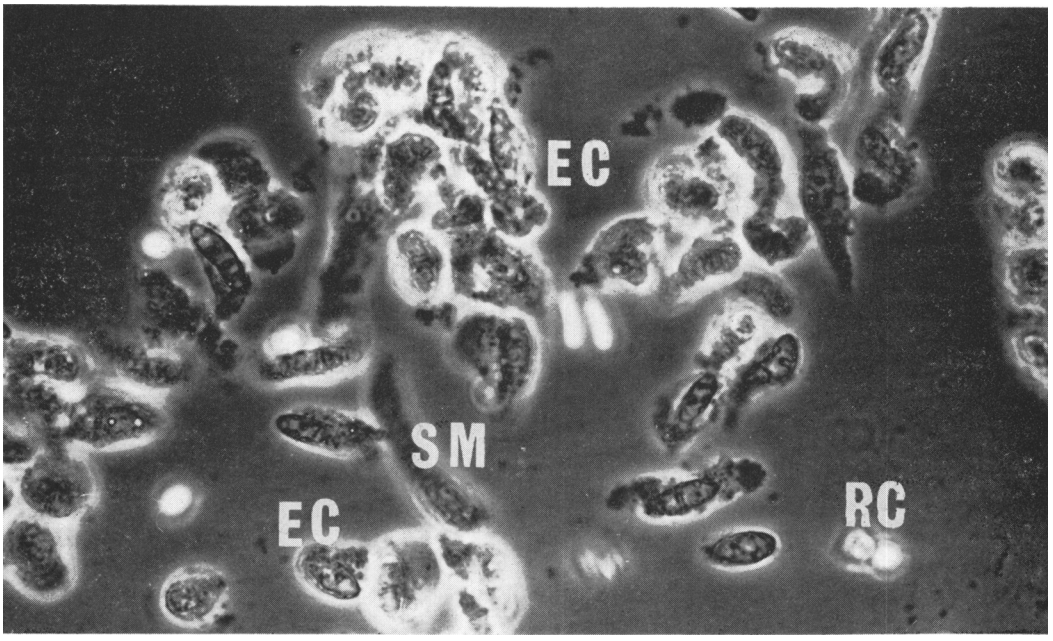

FIG. 1. Cell suspension following trypsin washing of bovine thoracic aortic lumenal surface (phase, 686×).

was prepared in KRP at 4° using a motor-driven ground glass Potter–Elvehjem homogenizer. Enzyme containing supernatants were then prepared as described above. Again, storage at —20° never exceeded 2 wk.

Cell culture. A 0.1 ml aliquot of suspension of isolated cells was suspended in 2 ml of McCoys 5A medium (8) (Grand Island Biochemical Co., Grand Island, NY) containing 20% autologous serum. This was then added to a coverslip coated with rat tail collagen and housed in a Leighton tube. Following 4 days incubation at 37°, fresh medium was added. The culture was terminated 4 days later with 4% formalin and the coverslip was stained with toluidine blue.

Histamine forming capacity (HFC). The histidine decarboxylase activity was determined by a modification of the method of Levine and Watts (9). A 0.5 ml aliquot of supernate was added to 25 ml incubation flasks (Kontes Glass Co., Vineland, NJ) containing 1.2 ml phosphate buffer (pH 7.3), 0.1 ml of 1.0 m*M* pyridoxal-5-phosphate and 0.1 ml of 1.0 m*M* streptomyocin sulfate. An enzyme blank for each sample was prepared as above, but in addition contained 1.0 ml of 1.0 *N* perchloric acid. Following 10 min shaking at 37° in a Dubnoff metabolic shaker, 0.1 ml ^{14}C-L-histidine (carboxyl-labeled) with specific activity of 1.0 μCi/ml (New England Nuclear Corp., Boston, MA), was added and the flask was sealed with a multiple dose rubber stopper containing a Kontes plastic center well holding a 2.3 cm Whatman paper scintillation pad. After 1 hr 55 min of incubation at 37°, 0.2 ml of 1.0 *M* hydroxide of Hyamine in methanol (New England Nuclear) was injected into the center well, and 5 min later the reaction was stopped by addition of 1.0 ml of 1.0 *N* perchloric acid into the incubation solution. The flasks were then shaken for 45 min at room temperature for trapping evolved $^{14}CO_2$. Finally the center wells were added to scintillation vials containing 15 ml of toluene scintillation fluorophor and the radioactivity counted in a Unilux II scintillation spectrometer (Nuclear Chicago Corp., Chicago, IL).

Results. Figure 1 is a phase photomicrograph (686×) of cells present in the pooled suspension obtained following the described trypsin treatment. While occasional red cells (RC), smooth muscle cells (SM), and cell fragments are present, it is clearly evident that over 90% of the cells within the suspen-

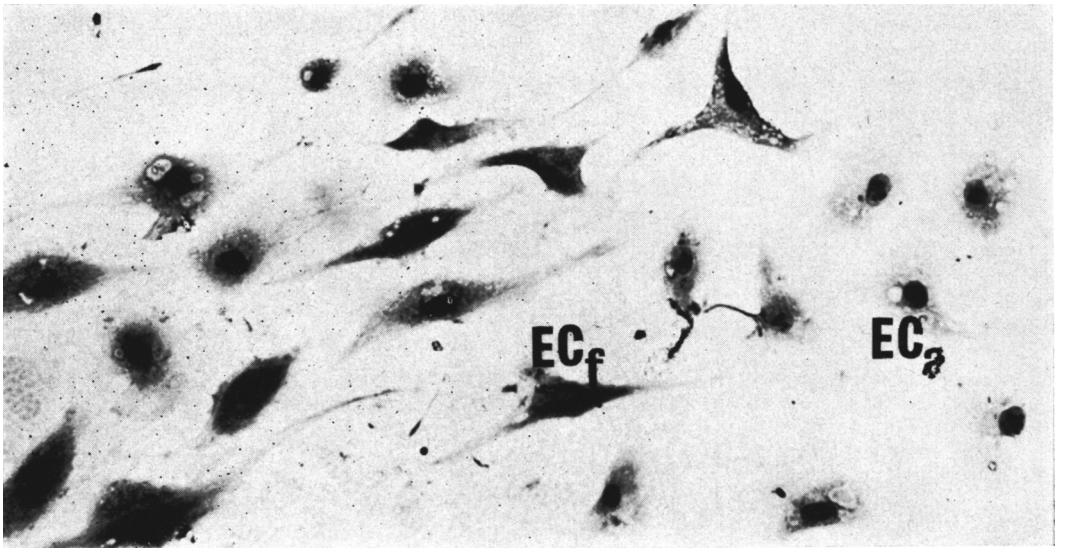

FIG. 2. Endothelial cells cultured on rat tail collagen following isolation by luminal enzymatic trypsinization (phase, 252 ×).

sion are endothelial cells (EC).

The appearance of this suspension following 8 days of culture is shown in Fig. 2. Inspection of this photomicrograph indicates 2 cell populations: fibrophilic endothelial cells (EC_f), and atherophilic endothelial cells (EC_a) characterized by the presence of prominent vacuoles. On a relative scale, 30% of the culture population had the appearance of atherophilic endothelium and 70% fibrophilic endothelium. The appearance of these cells following 8 days of culture clearly indicates the trypsin treatment yielded viable endothelium.

The HFC of both isolated endothelial cells and their paired intima–media homogenates is given in Table I. Each value is the mean of duplicate HFC determinations for cell suspensions or intima–media preparations from a given animal; group means, together with their respective standard errors, are given at the bottom of the table. These show an endothelial HFC of 4371 ± 806 dpm/mg protein and an intima–media HFC of 314 ± 46 dpm/mg protein. Variance analysis followed by Tukey's Least Significant Difference test (10) indicates this difference is highly significant ($p < .01$).

Initially additional HFC determinations were also performed on the pellet fraction of the homogenate obtained following centrifugation. Since the activity of the pellet fraction was in all cases not significantly different from background activity, the extraction method employed for this enzyme was essentially complete and confirmed work of others (7, 9, 11, 12).

Discussion. The morphological and functional characteristics of arterial endothelium are similar to those of continuous-type capillaries (14, 15). Similarly, arterial endothelium occupies a key role in the regulation of

TABLE I. Histamine Forming Capacity (HFC)[a] of Bovine Aortic Endothelium and Intima–Media.

Animal no.	Endothelium	Intima–Media
1	4575	304
2	2055	271
3	7176	492
4	6494	399
5	3926	203
6	1832	216
7	2028	—
8	6885	—
Mean	4371[b]	314
SEM	±806	±46

[a] Dpm/mg protein.

[b] Difference between wall components significant ($p < .01$, Tukey's test).

arterial wall permeability similar to the role of the combined endothelium and precapillary sphincters in the microcirculation. Thus, it is reasonable to assume that the HD activity of large artery endothelium should be similar to that of endothelium in the microcirculation. The results of this investigation clearly show that under these *in vitro* assay conditions the HD activity of bovine aortic endothelium is approximately 15× greater than that of subjacent intima–media.

It is obvious that the HFC of both the pooled cell suspension and the intima–media reflects the sum of individual HD activities of the various cells found within these two fractions. Histological studies of untrypsinized tissue employing either theonine or neutral red counterstained with hematoxylin showed a few granulated mast cells within the adventitia. None are seen following the adventitial stripping procedure employed prior to trypsinization. Thus, the HFC of the pooled cell suspension is a reasonably quantitative measurement of the HD activity of the endothelium, while that of subjacent intima–media reflects the HD activity of smooth muscle.

To determine whether trypsin treatment itself might contribute to the difference in observed HD activity between wall components, the HFC of rectangular specimens of intima–media devoid of endothelium and washed with trypsin–KRP solution were also determined. No significant difference in HFC was noted between trypsin-treated and control intima–media preparations. In addition, the trypsin treatment failed to produce mast cell degranulation in those few cells located at the adventitial–medial junction. While trypsin, like dextran, is a potent mast cell histamine releasing agent, the lack of its effect on mast cells using these procedures can undoubtedly be explained by lack of trypsin diffusion into the inner wall. This is not surprising in view of the relatively short time the tissue was exposed to the trypsin solution. In addition, trypsinization did not alter the relative activity ratio of HD between the endothelium and intima–media. This lack of trypsin effect, as well as that of dextran and other histamine releasing agents on histamine synthesis, has been previously demonstrated (1).

The results of the present investigation give, to our knowledge, the first experimental verification of Schayer's premise that the endothelium is the major site of histamine synthesis within the vascular system under essentially physiological conditions.

Summary. The HFC of bovine aortic endothelial cells was compared to that of subjacent intima–media homogenates. Results indicated that endothelial HFC, and thus the endothelial histidine decarboxylase system, was approximately 15× greater than that of the intima–media.

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Received June 7, 1972. P.S.E.B.M., 1972, Vol. 141.