

Aortic Histamine Synthesis in Experimental Neurogenic Hypertension (38714)

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Within the past few years it has become increasingly recognized and accepted that the arterial endothelium is sensitive to conditions imposed both by blood flow and blood pressure, that the endothelium actively regulates large artery transmural permeability, and that alterations in permeability are consistently produced by elevations in either shearing stresses or by the complex elevation of stresses which accompany the hypertensive state (1-5).

Our laboratory has been examining those metabolic mechanisms through which artery wall permeability is regulated and which are likewise capable of increasing transmural permeability in response to elevated intravascular stresses. Specifically we have been examining histamine synthesis within both the aortic wall and isolated wall components, in as much as the inflammatory nature of hypertensive aortic lesions has long been recognized and the effects of exogenous histamine on vascular permeability are well documented (6). In previous studies involving bovine, rabbit and rat aortas we have reported that histamine synthesis, mediated by the nonmast cell mammalian L-histidine decarboxylase system (EC 4.1.1.22), hereafter referred to as histidine decarboxylase (HD), is several orders of magnitude higher in aortic endothelium than in the subjacent media (7), that histamine synthesis is increased when either aortas in situ or primary cultures of endothelial cells are exposed to elevated shearing stresses (8, 9). Most recently we have shown that aortic histamine synthesis is increased in rats rendered hypertensive by abdominal aortic occlusion (10), a procedure described by Rojo-Ortego and Genest (11).

The present study has been designed to determine whether thoracic aortic histamine synthesis is likewise increased under a condition of short-term, transient neurogenic hypertension. The intent of this study is thus

to determine whether it is the elevated pressure per se or the surgical trauma to which the aorta is subjected by the procedures involved in aortic occlusion which is responsible for this elevation in aortic histamine synthesis.

Methods. Male Wistar rats, 350-450 g body weight, were divided into two experimental groups, i.e., a group consisting of animals previously subjected to right carotid artery ligation and left carotid artery coarctation, and a group additionally subjected to adrenalectomy. Corresponding control groups consisted of paired sham operated, unoperated, and adrenalectomized animals, with at least eight animals per treatment group.

Animals were sacrificed at 1-8 days after surgical treatment. At least 5 days prior to surgery all rats were housed in stainless-steel cages under controlled environmental conditions ($24 \pm 0.16^\circ$, 12 hr photoperiod, eight air changes per 24 hr) and were fed Purina Rat Chow and water ad lib. Animals subjected to any surgical procedure were fasted and given 5.3 mg/100 ml tetracycline in tap water 24 hr preoperatively. After surgery animals were again fed ad lib. and maintained on tetracycline; animals which were subjected to adrenalectomy were given 2% saline instead of tap water.

Experimental neurogenic hypertension was induced via partial occlusion of the left and total occlusion of the right common carotid arteries 5 mm above the sternal manubrium. The final external diameter of the left carotid was 0.1 mm, created by ligation around a stainless-steel probe of that diameter, followed immediately by probe withdrawal. In sham-operated control animals, the carotids were exposed and manipulated but not ligated. Bilateral adrenalectomy was performed through a midline laparotomy; in corresponding sham animals the adrenals were exposed but not subjected to manipu-

lation. All surgical procedures were performed under ether anesthesia.

Upon cessation of the designated post-operative time period, blood pressure of each animal was obtained under light ether anesthesia via abdominal aortic cannulation. The thoracic aorta, as delimited by the region between the diaphragm and left subclavian artery, was excised and perfused with saline to remove adhering blood. It was then stripped of the periadventitial sheath, and the remaining tissue was weighed and homogenized at 4° in 3.0 ml of 0.2 M potassium phosphate buffer (pH 7.4) containing 6 µg histamine dihydrochloride, 2.8 µg aminoguanidine sulfate and 0.66 mg glucose. The resulting homogenate was assayed for histidine decarboxylase activity using the ¹⁴C-L histidine (ring labeled) isotope dilution procedure of Schayer (12). Samples were counted on a Unilux II scintillation spectrometer (Nuclear Chicago), and the results were expressed as counts per minute per 100 mg benzenesulfonylhistamine (cpm/100 mg BSH).

Results. Animals were considered hypertensive if their systolic pressures, as recorded via abdominal aortic cannulation, were 140 mmHg or more. As no significant differences in blood pressure were noted either among any of the control groups or among the experimental groups, these data were pooled. The mean systolic pressure of pooled control animals was 118 ± 2.5 mmHg, while those of animals subjected to the carotid ligation-coarctation procedure for 1 and 8 days were 151 ± 2.4 mmHg and 133 ± 6.8 mmHg, respectively. Since blood pressure measured under ether anesthesia is at best highly variable, heart weights were used for additional evaluation of the extent of hypertension produced by the experimental procedures employed. The mean control heart weight/body weight ratio was 2.6 ± 0.06 × 10⁻³, while those of the 1- and 8-day treatment groups were 2.9 ± 0.06 × 10⁻³ and 2.6 ± 0.06 × 10⁻³, respectively. With respect to blood pressure, the mean systolic pressure of each experimental treatment group was significantly higher (*P* < 0.05) than that of control. In the case of heart weight/body weight ratios, only the mean ratio of the

TABLE I. THORACIC AORTIC HISTAMINE-FORMING CAPACITY (HFC^a) OF RATS SUBJECTED TO CAROTID SINUS ISCHEMIA^b AND BILATERAL ADRENALECTOMY FOR 24 HR.

Rat No.	Unoperated	Sham	Adrenalectomized	Carotid sinus ischemia	Carotid sinus ischemia and adrenalectomy
1	48	145	75	113	291
2	175	49	125	155	116
3	87	43	45	150	150
4	23	94	174	245	136
5	146	102	77	243	212
6	87	197	89	216	216
7	87	192	46	256	307
8	154	108	78	271	255
Mean	101	116	97	194 ^c	210 ^c
SEM	±19	±20	±15	±21	±25

^a HFC = counts per minute per 100 mg benzenesulfonylhistamine (cpm/100 mg BSH).

^b Carotid sinus ischemia produced by ligation of right and coarctation of left common carotid arteries 5 mm cephalad to sternal manubrium.

^c Difference from control significant (*P* < 0.05, Tukey's test).

1-day experimental treatment group was significantly (*P* < 0.05) higher than control.

Tables I and II present all data relative to the thoracic aortic HFC's for each individual animal in each control and experimental treatment group, together with respective group means and mean standard errors. As is indicated, no significant differences exist among sham-operated, unoperated, and adrenalectomized controls. As shown in Table I, the mean thoracic aortic histamine-forming capacities (HFC) of the 24-hr hypertensive group was 83% greater than that of the pooled control value, while the mean HFC of the hypertensive, adrenalectomized group was elevated 103% over the same pooled control values. No significant differences exist between either of the experimental treatment group means. Similarly, the mean HFC of thoracic aortas from animals subjected to the carotid ligation-coarctation procedure for 8 days was 33% higher than control while the mean HFC of animals additionally subjected to bilateral adrenalectomy was 45% greater than that of the pooled control

TABLE II. THORACIC AORTIC HISTAMINE-FORMING CAPACITY (HFC^a) OF RATS SUBJECTED TO CAROTID SINUS ISCHEMIA^b AND BILATERAL ADRENAL-ECTOMY FOR 8 DAYS.

Rat No.	Unoper-ated	Sham	Adre-nalecto-mized	Carotid sinus ischemia	Carotid sinus ischemia and adre-nalectomy
1	48	159	144	114	100
2	175	48	142	107	224
3	87	48	160	125	167
4	23	99	33	88	132
5	146	60	111	193	169
6	87	31	53	130	101
7	87	156	123	132	97
8	154	140	101	178	172
Mean	109	92	108	133 ^c	145 ^c
SEM	±16	±18	±16	±12	±16

^a HFC = counts per minute per 100 mg benzene-sulfonylhistamine (cpm/100 mg BSH).

^b Carotid sinus ischemia produced by ligation of right and coarctation of left common carotid arteries 5 mm cephalad to sternal manubrium.

^c Difference from control significant ($P < 0.05$, Tukey's test).

mean. Both differences are significant ($P < 0.05$) with respect to control, significantly lower ($P < 0.05$) than those of the 1-day treatment groups, and not significant from each other.

Discussion. Krieger (14) has reported that over a 2-wk period after resection of both carotid bifurcations and cervical sympathetic-depressor trunks mean blood pressure shows a phasic change from 175 mm Hg to 125 mm Hg. The mean systolic pressure of the rats subjected to the carotid artery ligation:coarctation procedure for 8 days was approximately 17% lower than that of animals in the 1-day postoperative treatment group. While the procedures employed in the present study differ from those of Krieger, it is apparent that the method used here produces a milder, shorter-term, transient hypertension than that observed by Krieger, and is presumably mediated through neurogenic mechanisms invoked by carotid sinus ischemia. The decline in mean systolic pressure over this 8-day period may be the result of baroreceptor adaptation (13, 14), or the development of collateral circulation to the

sinus region, as described by Iriuchijima and Hiroyuki (15). The lowered heart weight:body weight ratio of the 8-day treatment group further substantiates the transitory nature of this hypertensive model.

The results of the present investigation indicate that aortic HD activity, and hence aortic histamine synthesis, increases as a result of transient, neurogenic hypertension. Significantly, the smaller increase in aortic HFC seen after 8 days of the carotid ligation:coarctation procedure is consistent with both the lowered mean systolic pressure and heart weight:body weight ratios observed at this time. In this respect, the mean systolic pressure of the 8-day treatment group, while below the arbitrarily defined minimal hypertensive pressure of 140 mmHg (11), is still elevated 75% with respect to the paired controls. That the HFC is still partially elevated at this time is most likely indicative of the continuance of inflammatory reactions occurring within the aorta in response to the elevated pressure. This is supported by a previous study in which we have shown that temporal changes in aortic HFC occur within a 6-hr period (10), thus minimizing any significant carry-over in histamine synthesis induced by the initial hypertension as seen in the 1-day treatment group. These data also indicate that at least in this hypertensive model the aortic HFC is not significantly influenced by adrenal responses. We feel that this is important to note, since glucocorticoids are apparent antagonists of effects of the HD system while catecholamines both augment HD activity (16) and produce morphological aortic endothelial changes similar to those of histamine (3, 17).

It is well established that the endothelium is the primary regulator of large artery permeability. Arterial endothelium displays properties similar to those of continuous-type capillaries; in the latter, permeability regulation is presumably achieved through control of the two functional pore systems (18, 19). The large pores are formed by plasmalemmal vesicles and have a functional diameter of 250–500 Å; the small pores, having a diameter of 90 Å, are formed by interendothelial junctions. Based on alterations in distribution and uptake of ferritin

(110 Å diameter) and colloidal carbon (250–500 Å diameter), Hüttner *et al.* (5) have shown that alterations in these pore systems occur in hypertensive rats. Noting the presence of vesicular ferritin and interendothelial colloidal carbon, they have suggested that hypertensive aortic wall permeability increases may be caused both by increased pinocytotic activity and by a chemically mediated, transitory widening of interendothelial junctions. Their observations are consistent with findings of Suzuki *et al.* (20) and Majno and Palade (21) who, respectively, have shown increased permeability of the aortic wall to fibrin and chylomicra in hypertension. Furthermore, Majno and Palade have also correlated increased permeability with endothelial cell contraction and the formation of distinct interendothelial cell gaps, and have shown that contraction can be induced by injection of histamine (17, 21).

Since the aorta synthesizes a vasoactive amine capable of inducing endothelial contraction, at least when administered exogenously, it is our working hypothesis that alterations in aortic transmural permeability which consistently occur under conditions of elevated hemodynamic stresses may be, at least in part, mediated through increased activity of the aortic HD system. The present study when considered in light of the previously reported increase in aortic histamine synthesis as a consequence of mechanically induced hypertension (10), supports this premise, since in these two diverse hypertensive models one common aortic response is increased histamine synthesis. Further studies which involve examining interrelationships between blood pressure, histamine synthesis, and aortic wall permeability to plasma proteins and water are currently in progress to more clearly define and substantiate the role of the aortic HD system in regulation of transmural permeability and the alterations in permeability consistently associated with both hypertension and atherogenesis.

Summary. Aortic histamine synthesis of normotensive rats has been compared to that of rats rendered hypertensive via production carotid sinus ischemia and to animals addi-

tionally subjected to bilateral adrenalectomy. Results indicate that the aortic histamine-forming capacities (HFC's) of rats sacrificed 24 hr after surgical treatment were elevated 83% and 103% for hypertensive rats and those additionally subjected to adrenalectomy, respectively. At an 8-day postoperative interval, the respective aortic HFC's were elevated over control by 33% and 45%, although at this time interval animals were not considered hypertensive. This study thus offers suggestive evidence that hypertension causes increased vascular permeability by increasing the histamine-forming capacity of the vessel wall.

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