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Studies on Aortic Histamine Synthesis in Experimental Diabetes (41097)

STEPHEN G. GALLIK AND THEODORE M. HOLLIS

Department of Biology, 208 Mueller Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16802

Abstract. Aortic histamine synthesis and aortic permeability to plasma-derived bovine serum albumin conjugated to fluorescein isothiocyanate (FITCBSA) have been examined in rats held for 2- and 4-week periods following diagnosis of overt diabetes after subtotal pancreatectomy. Results indicate that aortic histamine synthesis, as measured through determination of aortic histidine decarboxylase (HD) activity in intima-media preparations, was increased by 15% at the end of the 4-week period of uncontrolled diabetes, while aortic albumin permeability increased 36% over corresponding control values. These results are similar to changes in aortic histamine synthesis and albumin permeability observed in a variety of other atherogenic situations, thus suggesting that increased aortic histamine synthesis may be involved in the known increased susceptibility of diabetes to atherosclerotic vascular disease.

Epidemiological studies have indicated that atherosclerosis is a major complication of diabetes mellitus (see Ref. (1) for current summary); additionally, numerous reports indicate that atherosclerotic vascular disease is more prevalent among diabetics than nondiabetics, and is responsible for a significantly higher mortality rate of these individuals (2, 3). Indeed, this relationship is so well established that diabetes constitutes one independent risk factor of atherosclerosis (3, 4). However, mechanisms responsible for the atherogenic nature of diabetes are still uncertain. While numerous studies have been directed toward characterizing alterations in protein, lipid, and carbohydrate metabolism in liver, adipose tissue, and muscle, as well as whole body effects of such alterations in the diabetic state (3–5), only a few studies have specifically addressed metabolic alterations occurring in vascular tissue as a direct result of diabetes. One important such study regarding such vessel wall alterations is that of Wolinsky *et al.* (6), who have shown that activity of a number of aortic smooth muscle hydrolases is impaired in untreated diabetic animals.

The present study represents an initial investigation into the determination of whether or not alterations in aortic histamine synthesis occur under diabetic conditions. The rationale behind this study is based on previous observations that arterial *de novo* histamine synthesis is increased under a variety of other atherogenic conditions, i.e., hypertension (7, 8), elevated shear stresses (9–12) and dietary-induced hypercholesteremia (13). In all cases, such increases in histamine synthesis have been intimately related to increases in aortic wall permeability to circulation albumen (13, 14), and with respect to hypercholesteremia, such increases are transient; the increase in histamine synthesis in this specific case is restricted to the endothelium (15, 16). If aortic histamine synthesis is increased in untreated diabetic animals, a similar situation resulting in increased wall uptake of circulating macromolecules might exist and might in turn represent one mechanism whereby increased low-density lipoprotein (LDL) is presented to underlying smooth muscle for subsequent clearance and metabolism. Such an increase in aortic histamine synthesis would thus represent a common response of the vascular wall to diabetes and to other conditions associated with atherosclerosis.

Methods. Male Wistar rats, having initial body weights of 135–175 g, were divided into four treatment groups. Two groups of experimental animals underwent subtotal pancreatectomy and, following attainment of diabetes, were maintained for either 2 or 4 weeks. Two groups of control animals

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underwent a sham operation and were held for the same times as those subjected to subtotal pancreatectomy.

The subtotal pancreatectomy was conducted under ether anesthesia using a modification of the method described by Scow (17). The sham operation consisted of visceral manipulation for approximately 30 min, the duration required to perform the subtotal pancreatomy. Following surgery, penicillin (100,000 U in 2 ml of sterile saline) was administered subcutaneously. During the immediate 2-hr postoperative period, the animals were kept warm by means of a heat lamp; during the first 8-hr postoperative period, food but not water was withheld from all animals. After recovery, animals were housed in stainless steel cages under controlled environmental conditions (24°, 14-hr photoperiod, 8 air changes/24 hr). All animals were maintained on Purina Rat Chow and water *ad lib*.

Two weeks following surgery, each animal was fasted for 12 hr, loaded with glucose (1.75 mg/kg body wt in a 25% solution, ip), and subjected to a 2-hr intraperitoneal glucose tolerance test (2-hr IPGTT). Plasma glucose was measured enzymatically (18) at weekly intervals until diabetes was evident using criteria of Seltzer (19), i.e., a fasting plasma concentration of 150 mg/dl and a 2-hr postinjection concentration of 180 mg/dl. Sham-operated controls were carried through the same number of IPGTTs as their initial weight-matched diabetic counterpart.

At the end of the experimental holding period, a final fasting blood sample was obtained for subsequent determination of plasma glucose concentration. Each animal was then injected with bovine serum albumin conjugated to fluorescein isothiocyanate (FITCBSA, 110 mg/kg in pH 7.4 PBS at a concentration of 50 mg/ml, jugular vein) for subsequent measurement of aortic transmural albumin uptake (20). Animals were killed by decapitation 1, 3, 6, and 12 hr after albumin injection.

The entire thoracic aorta was excised, placed in cold phosphate-buffered saline (PBS, pH 7.1, 4°), stripped of its periadventitial fat, perfused under low pressure with cold PBS to remove adhering blood, opened longitudinally, and weighed. Each aorta was homogenized in 20 vol of cold PBS (pH 7.1, 4°, 20 passes over a 2-min duration) using a motor-driven ground-glass pestle. The homogenate was centrifuged (15,000*g*, 4°, 20 min), and the resulting supernatant was stored in liquid nitrogen until determinations of histidine decarboxylase (HD) and the intrathoracic FITCBSA content was performed.

HD activity, expressed as histamine-forming capacity (HFC), was measured by modification of the method of Levine and Watts (21), as previously described (22). Briefly, this consists of diluting the thawed supernatant solution to 200 ml with PBS (pH 7.1) and incubating a 1.85-ml aliquot of it in a 25-ml incubation flask containing 0.05 ml of 1 m*M* pyridoxal-5-phosphate and 0.1 ml of ^{14}C-L-histidine (carboxyl labeled, New England Nuclear). The final specific activity of this isotope was 1 μCi/ml. $^{14}CO_2$ liberated over a 1.75-hr incubation period at 37° was trapped on fluted filter paper treated with 1 *M* hyamine hydroxide. The reaction was terminated at 2 hr by adding 1 ml of 10% trichloroacetic acid (TCA) to the reaction medium. This was followed by an additional 45-min incubation at ambient temperature to allow for absorption of liberated $^{14}CO_2$. Blanks containing supernatant solution previously treated with TCA. Activity was measured in a Packard Tricarb liquid scintillation counter, corrected for background and counting efficiency, and expressed as disintegrations per minute per milligram of aorta (dpm/mg, wet wt).

Aortic albumin uptake was measured using procedures developed in this laboratory (20). Briefly, BSA was conjugated to FITC by a modification of Nairn's method. A 0.3-ml aliquot of aortic supernatant was transferred to a microcuvette, and fluorescence was measured in an SF 103 fluorescence spectrophotometer (Baird-Atomic) using wavelengths (in nm) of 490 excitation and 520 emission. Fluorescence readings were compared to those obtained from a standard curve, and the amount of FITCBSA per sample was determined and corrected

for dilution. Results were expressed as micrograms of FITCBSA per gram of aorta, wet weight).

Results. Rats subjected to subtotal pancreatectomy typically developed diabetes associated with increased food and water intake, polydipsia, and increased urine excretion 1-½ to 2 weeks after surgery. Data in Table I indicate that 2 and 4 weeks following induction of overt diabetes, diabetic rats showed a significant reduction in the rate of weight gain, elevated fasting, and 2-hr IPGTT glucose concentrations. All differences from controls are significant ($P < 0.001$), but no significant differences exist between the two diabetic treatment groups.

Data of aortic FITCBSA contents and corresponding mean HFCs for the same aortas (Table II) indicate that animals rendered diabetic for a 2-week period showed a 41% increase in intrathoracic FITCBSA accumulation and a 13% increase in aortic histamine formation, as measured by HFC determinations. Similarly, aortas from rats of the 4-week diabetic treatment group showed a 17% increase in aortic histamine synthesis and a 57% increase in intraaortic FITCBSA. All differences are significant from corresponding control values ($P < 0.05$).

Discussion. In this study rats were rendered diabetic by removal of at least 95% of the pancreas. This procedure was chosen for these initial studies in order to avoid the use of diabetogenic drugs which may have a direct effect on aortic histamine synthesis. However, this particular model is exceedingly complex, involving significant loss not only of beta cell function but also that of most alpha cells and exocrine function. Thus the possibility exists that alterations in histamine synthesis might be a result of complications induced by decreased digestive and absorptive capacities of the gut. Body weight data clearly indicate that the rats subjected to pancreatectomy gained weight, but that their final body weight was significantly lower than the final body weight of sham-operated animals. That such a reduced rate of growth of these animals is an effect of diabetes rather than malnutrition is supported by striking similarities between our data and body weight data of Wolinsky *et al.* (6), who used Streptozotocin, and by the data of Uram *et al.* (24), who showed that in the rat only 1% of the pancreas is needed to achieve normal or near normal digestion and absorption. In our studies at least 3% of the pancreas was left intact in all animals.

Results of this study indicate that in the diabetic state, as achieved by subtotal pancreatectomy, there is a significant increase in aortic histamine synthesis. We interpret this finding to suggest that a large vessel equivalent of the microcirculatory prolonged phase of inflammation may be occurring, a response which is mediated at least in part by the inducible histidine decarboxylase system (25). Such an increase in histamine synthesis is consistent with responses of the aortic HD system to other known atherogenic factors. For example, under *in situ* conditions using perfused rabbit aortas, as well as in dog aortas *in vivo*, there is a linear relationship between the

TABLE I. CHARACTERISTICS OF ANIMAL GROUPS

Characteristic	2-Week group		4-Week group	
	Control	Diabetic	Control	Diabetic
Initial body weight (g)	156 ± 2.6	157 ± 2.6	147 ± 2.9	159 ± 2.5
Final body weight (g)	396 ± 9.3	250 ± 10.4[a]	430 ± 8.1	251 ± 9.5[a]
Aortic weight (g)	171 ± 3.4	131 ± 4.1[a]	170 ± 3.7	134 ± 5.0[a]
Fasting plasma glucose (mg/dl)	98 ± 4.6	299 ± 12.4[a]	94 ± 4.3	241 ± 13.8[a]
2-hr IPGTT (mg/dl)	122 ± 6.2	416 ± 17.9[a]	115 ± 6.0	405 ± 20.3[a]

Note. Each treatment group contained 20 animals ($n = 20$); all values are expressed as means ± SE.
[a] Denotes significant difference from corresponding control value ($P < 0.001$, Student's t test). Diabetes was induced by subtotal pancreatectomy. Controls were subjected to sham operation.

TABLE II. THORACIC AORTIC HISTAMINE FORMING CAPACITY (HFC) AND INTRAAORTIC CONTENT OF BOVINE SERUM ALBUMIN CONJUGATED TO FLUORESCEIN ISOTHIOCYANATE (FITCBSA) OF DIABETIC RATS

Group	HFC (dpm/gm aorta)	FITCBSA content (μg/gm aorta)
2-Week control	49,949 ± 695	46.5 ± 2.6
2-Week diabetic	56,208 ± 766[a]	65.5 ± 3.2[a]
4-Week control	49,537 ± 482	43.5 ± 2.0
4-Week diabetic	57,979 ± 738[a]	68.4 ± 4.4[a]

Note. Each treatment group contained 20 animals ($n = 20$); all values are expressed as means ± SE. Aortic FITCBSA content was measured under steady-state conditions (see text), with diabetes being induced by subtotal pancreatectomy.

[a] Denotes significant difference from corresponding control value ($P < 0.01$, Student's t test). Results standardized on wet weights.

applied shear stress and the resultant aortic HFC (10–12). The same type of response is observed when cultured endothelial cells are subjected to varied intensity shear stress intensities (26). Such an increase in aortic HFC is likewise seen in both experimental neurogenic (7) and mechanical hypertension (8) as well as in aortas from rabbits subjected to short-term cholesterol feeding (13). In the latter case, the increase in HFC is transient, occurring prior to the occurrence of any visible atherosclerotic plaques (15, 16), and is of the same order of magnitude as in the present investigation; the same applies to aortic FITCBSA uptake (27).

We believe that this initial study has important implications with respect to the atherogenic potential of diabetes mellitus. Specifically, we (14) recently reported that partial inhibition of the aortic histidine decarboxylase system of α-hydrazinohistidine reduces the severity of atherosclerosis in cholesterol-fed rabbits which were not given α-hydrazinohistidine, with a significant correlation between the aortic HFC and other albumin uptake. On the basis of this and other studies, we proposed that the aortic HD system may be one of the determinants of the changes in vascular permeability in early atherogenesis. That endothelial resistance to macromolecules in diabetes is reduced is indicated by the increased content of plasma-derived FITCBSA in diabetic aortas, even though the plasma FITCBSA concentration was not increased. Thus, permeability of the vascular wall to circulating macromolecules (such as low-density lipoproteins) may also be increased at the same time that the concentration of LDL is elevated, increasing the supply of substrate underlying vascular smooth muscle cells. Moreover, Wolinsky *et al.* (6) have shown that lysosomal acid cholesteryl esterase acitivty in diabetic rats is markedly reduced. Thus the ability of smooth muscle to clear incoming LDL would be impaired just as more LDL is being presented for clearance. We speculate that an increase synthesis of histamine by the aorta may be involved in this process and we believe that future studies of relationships between diabetes and atherosclerosis should include examinations of aortic vasoactive amine metabolism. Such studies are in progress in our laboratory.

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