**Articles**

**Retinal Histamine Synthesis Is Increased in Experimental Diabetes**

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We examined retinal de novo histamine synthesis mediated by retinal histidine decarboxylase in normal and streptozotocin-diabetic male, Sprague Dawley rats that were diabetic for 21 days. We also examined effects of insulin and alpha-hydrazinohistidine (αHH) treatments on retinal histamine synthesis in this diabetic model. αHH is a specific inhibitor of histidine decarboxylase. Results indicate that the retina contains an active histidine decarboxylase enzyme system, and that in streptozotocin diabetes retinal histamine synthesis is increased 197%. Both insulin and αHH independently reverse and normalize retinal histamine synthesis. These data thus indicate that the retinal inducible histamine pool is increased in experimental diabetes, and that insulin is an important modulator of retinal histamine metabolism. This newly described retinal metabolic alteration may be one factor responsible for increased retinal vascular permeability in diabetic retinopathy. Invest Ophthalmol Vis Sci 29:1201-1204, 1988

Diabetes mellitus is accompanied by a wide array of metabolic disorders. Numerous clinical problems of diabetics are associated with specific aberrations in one or more pathways associated with altered protein, carbohydrate and lipid metabolism. Recently Orlidge and Hollis1 and Hollis et al2 have reported that in experimental diabetes there are marked changes occurring in vascular histamine metabolism as well. Specifically, in streptozotocin-diabetic rats, aortic endothelial and smooth muscle cell histamine synthesis is increased and histamine catabolism is decreased, which results in a concomitant increase in intracellular histamine content.1 Plasma histamine concentrations are doubled in these same animals, and reflect altered systemic histamine metabolism.2 Changes in histamine metabolism can be reversed by insulin therapy1 or by treatment with alpha-hydrazinohistidine (αHH, 2, 4), a relatively specific inhibitor of histidine decarboxylase (HD).

Increased histamine synthesis in diabetes has been tightly correlated with changes in aortic albumin permeability.2,4 Since macroangiopathies affecting vessels such as the aorta in experimental diabetes are associated with elevated histamine synthesis,1,2,4 these degenerative lesions may reflect a histamine-mediated vascular response. In addition to degenerative effects on large vessels, diabetes is also associated with microvessel pathology, especially in the eyes and kidneys. While macroangiopathy has been associated with elevated histamine synthesis,1,2,4 to date no such correlation has been made with the microangiopathies, but both conditions involve early increases in vascular membrane macromolecule permeability. To this end, in the present study retinal histamine synthesis has been examined in normal and streptozotocin-diabetic rats. Results of the present study indicate that retinal histamine synthesis is increased in experimental diabetes, and that the magnitude of this increase is similar to that occurring in the aorta. These results suggest that de novo histamine synthesis may mediate initial functional degenerative alterations observed in diabetic retinopathy.

Materials and Methods

A total of 57 male Wistar rats having initial body weights of 160–180 g were used in this study, with all experiments meeting the ARVO Resolution on Use of Animals in Research. Diabetes was induced in 36
of these animals by a single jugular vein injection of streptozotocin (Streptozotocin, 65 mg/kg, Sigma Chemical Co., St. Louis, MO) in citrated, phosphate buffered saline (PBS, pH 4.5) under anesthesia (ketamine HCl, 50 mg/kg, Bristol Lab., Syracuse, NY; xylazine, 5 mg/kg, Haver-Lockhart, Shawnee, KS). Animals were housed individually in hanging metal cages and given standard laboratory chow and water ad libitum for 21 days. Housing conditions were maintained at 25°C, 50% humidity, and a 6 AM–6 PM photoperiod. Nonfasting blood glucose determinations were made prior to injection and weekly following streptozotocin injection for 3 weeks using a YSI 23A glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Control animals received citrated PBS and were treated as above. In addition, body weights, urine glucose concentrations, urine volumes, and individual animal’s fluid intake, were also measured weekly for a 24 hr period.

On day 17, diabetic animals were divided into three subgroups each containing a minimum of five animals. Alzet model 2001 osmotic minipumps (Alzet, Palo Alto, CA) having a mean flow rate of 1 ml/hr and containing alpha-hydrazinohistidine (αHH, Regis Chemical Co., Morton Grove, IL) dissolved in Dulbecco’s saline were implanted into the intraperitoneal cavity of one subgroup. This group received αHH at a dosage of 50 mg/kg/24 hr, a dose known to prevent or reverse increased aortic albumin permeability characteristic in streptozotocin-diabetic rats. The remaining two diabetic subgroups were sham-operated, and one of these subgroups received insulin (5–7 U, NPH Iletin I isophane insulin suspension, Eli Lilly and Co., Indianapolis, IN) daily at 4:00 PM. Urine and plasma glucose concentrations were measured during this 5-day treatment period just prior to insulin administration in order to adjust insulin dosages to maintain euglycemia (80–130 mg/dl).

On the 22nd day of the holding period, animals were anesthetized with ketamine and xylazine as described above, decapitated, and both eyes removed by enucleation and placed in ice-cold PBS (pH 7.4). A small incision was made through the eye parallel to and slightly anterior to its equatorial plane. This initial incision was then extended along the ora serrata around the eye’s circumference, separating the eye into anterior and posterior halves. The neurosensory retina was gently teased from the retinal pigment epithelium, cut at its point of attachment with the optic nerve, and pooled with the other retina from that animal before being homogenized in 0.8 ml PBS (0.1 M, pH 7.4) containing Triton X-100 (0.1% w/v, Sigma Chemical Co.). Retinal homogenates were then centrifuged at 6,700 g (15 min, 25°C, and duplicate samples were stored in 400 ml polypropylene tubes at −70°C.

**Histidine Decarboxylase Activity**

Retinal histidine decarboxylase activity was determined using a modification of an enzyme isotopic assay of histamine described by Taylor and Snyder. Tissue samples were incubated with [3H]-methyl-S-adenosyl-L-methionine ([3H-SAMe, 20 Ci/ml, ICN, Irvine, CA] and histamine-N-methyltransferase (HMT, E.C. 2.1.1.8) prepared from rat kidneys using procedures described by Shaff and Beaven. The details of this assay, together with modifications, have been described elsewhere. Histidine decarboxylase activity was expressed as ng histamine formed/hr/mg protein. Protein determinations were made using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA).

**Statistical Analyses**

Comparison of group means was performed using analysis of variance followed by application of Duncan’s new multiple range test. A significance level of 0.05 was used to define a significant difference.

**Results**

Whole body metabolic characteristics of each of the four treatment groups are presented in Table 1. Initial body weights were similar for all treatment groups, ranging between 199 g and 223 g at the time of streptozotocin injection. There were no significant differences between initial animal body weights among groups (P > 0.05) and final body weights among these same animal groups (P > 0.05). Nonfasting plasma glucose concentrations were significantly elevated (P < 0.05) in all diabetic animals, as was 24 hr water intake and urine output values. Additionally, urine glucose concentrations equaled or exceeded 2000 mg/dl in diabetic animals, while there was no detectable glucose in urine of control animals.

Mean retinal histidine decarboxylase (HD) activities for each of the treatment groups are presented in Figure 1. These data show that HD activity was significantly elevated (P < 0.05) in untreated diabetic animals, an increase of 197% over control values. HD activity in the insulin-treated diabetic animals was not significantly different from control (P > 0.05); similarly, retinal HD activity in the αHH-treated diabetic animals was not significantly different from control animals (P > 0.05), despite the persistence of hyperglycemia and other manifestations of experimental diabetes. Likewise, αHH had no significant effect on retinal HD activity of control animals.
Table 1. Characteristics of animal groups

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Body weights (g)</th>
<th>Plasma glucose (mg/dl)</th>
<th>Water intake (ml/24 h)</th>
<th>Urine output (ml/24 h)</th>
<th>Urine glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control (n = 10)</td>
<td>209 ± 2</td>
<td>315 ± 11</td>
<td>121 ± 2</td>
<td>42 ± 3</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Control-αHH (n = 8)</td>
<td>199 ± 3</td>
<td>307 ± 8</td>
<td>123 ± 1</td>
<td>47 ± 5</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Diabetic (n = 10)</td>
<td>205 ± 4</td>
<td>301 ± 9</td>
<td>416 ± 26*</td>
<td>144 ± 24*</td>
<td>105 ± 23*</td>
</tr>
<tr>
<td>Diabetic-αHH (n = 10)</td>
<td>209 ± 4</td>
<td>303 ± 9</td>
<td>436 ± 29*</td>
<td>179 ± 27*</td>
<td>140 ± 14*</td>
</tr>
<tr>
<td>Diabetic-insulin (n = 12)</td>
<td>223 ± 4</td>
<td>303 ± 11</td>
<td>452 ± 21</td>
<td>158 ± 22*</td>
<td>129 ± 22*</td>
</tr>
</tbody>
</table>

Diabetes induced by jugular vein injection of streptozotocin (65 mg/kg). Holding period was 21 days. αHH = α-hydrazinohistidine (50 mg/kg/24 hr) administered via subcutaneously implanted Alzet minipumps during days 17–21. Insulin (NPH Hletin, 5–7 U/24 hr) given daily at 4:00 PM via subcutaneous injection. All values are means ± mean standard errors. Numbers in ( ) denote group means following insulin treatment.

Thus, mean retinal HD activity was significantly elevated in untreated diabetic animals only. There were no differences in retinal HD activity between insulin-treated and αHH-treated diabetic animals (P > 0.05).

Discussion

Orlidge and Hollis reported that aortic endothelial and smooth muscle cell histidine decarboxylase activity is markedly elevated in streptozotocin diabetic rats, and that this change could be totally reversed by treatment of diabetic rats with insulin during the last 25% of the diabetic holding period. In a related study, increases in aortic histidine decarboxylase activity were completely reversed by administration of αHH. This treatment likewise reversed increased aortic albumin to macromolecules such as albumin. Similarly, plasma histamine concentrations were also normalized in diabetic animals by treatment with either insulin or with αHH. Results of the present study show an increase in retinal histidine decarboxylase activity in streptozotocin-diabetic rats. Both insulin and αHH, which modulate HD activity in the aorta, also inhibit retinal HD activity in this diabetic model.

Histamine exists in mammalian tissues in three different functional pools. Two of these pools have a slow turnover rate. The first of these represents histamine stored as a heparin-protein complex in se-
cretry granules of tissue mast cells. This preformed histamine pool has a half-life of 50 days and is rapidly depleted by histamine-liberating agents such as Compound 48/80. The second slow turnover pool is not subject to depletion by histamine-liberating agents and is therefore considered either to be of nonmast cell origin or from a different subclass of mast cells resistant to actions of these agents. Histamine located in this second slow-turnover pool is found in organs such as the heart, lungs, and stomach. The third histamine pool present in most tissues exhibits a rapid turnover of 3–6 hr. The magnitude of this pool can be dramatically altered by changes in rates of histamine synthesis mediated by histidine decarboxylase activity. It is this third pool that represents induced or nascent histamine formed in response to variously locally acting stimuli. The induced histamine pool is putatively associated with neurotransmitter function, regulation of gastric HCl secretion, tissue growth and repair, vascular permeability, and with mediating certain aspects of the prolonged phase of inflammation.

Results of this study clearly show that in streptozotocin-induced diabetes in the rat there is a marked increase in retinal histidine decarboxylase activity, therefore indicating that there is an expansion of the retinal inducible histamine pool. Insulin has been shown to be an important modulator of the HD system in vivo. In experimental diabetes, insulin deficiency is directly responsible for the observed expansion of the inducible histamine pool in the retina.

At present, it is uncertain as to what component or components in the retina are the source of this elevated histamine synthesis. For example, there is a growing body of evidence suggesting that histamine is a retinal neurotransmitter. Recently Nowak et al examined histamine distribution and localization within bovine and rabbit eyes. They reported that 90% of the histamine was contained in the 1000 g fraction composed of nervous tissue, inner and outer rod segments, mitochondria, and blood vessels and microvascular elements. They concluded that the two elements whose connection with histamine seems most probable are the microcirculation and rod segments. This, obviously, needs further clarification, and such studies are currently in progress. However, collectively these present data suggest that an increase in the retinal-induced histamine pool in diabetes may mediate blood-retinal barrier leakage through a prolonged type of inflammatory response.

Histamine is one putative intrinsic regulator of microcirculatory flow. Cerebral vessels, which share a common neuroectodermal origin with retinal vessels, respond to histamine infusion by increased permeability. Gulati et al have reported that metiamide, an H2-receptor antagonist, prevents blood-brain barrier (BBB) permeability increases to sodium fluorescein mediated by histamine infusion in mongrel dogs. Furthermore, Lorenzi et al have shown that BBB permeability is increased in streptozotocin diabetes. Therefore, it would appear highly probable that inductible, nonmast cell histamine may mediate blood-retinal barrier permeability in experimental diabetes.

Key words: retinal histamine, histidine decarboxylase, histamine synthesis, experimental diabetes, streptozotocin diabetes in rats

References