Reports

Hexitol Production by Canine Retinal Microvessels

Timothy S. Kern and Ronald L. Engerman

Hexitol-producing activity has been quantitated in microvessels isolated from retina and cerebral cortex of dogs, a species known to develop a retinopathy similar to that seen in diabetic patients. Both the retinal and cerebral microvessels produce galactitol from galactose, but at a rate several-fold less than that seen in canine lens epithelium. This hexitol-producing activity of the microvessels is not an artifact of erythrocyte contamination and can be inhibited by the aldose reductase inhibitor, Sorbinil. Invest Ophthalmol Vis Sci 26:382-384, 1985

Production and accumulation of the hexitols, sorbitol and galactitol, have been implicated in the pathogenesis of several complications of diabetes and galactosemia, including cataract and nerve conduction defects. Hexitol production might be related also to the development of the retinopathy that occurs in diabetes and in experimental galactosemia. It is conceivable that hexitol is produced within microvascular cells during periods of elevated blood sugar, and that accumulation of the hexitol results in cell damage and, eventually, retinopathy.

In the present study, the ability of retinal and cerebral microvessels to produce and accumulate galactitol from galactose has been examined. Microvessels were isolated for this purpose from dogs, a species known to develop a retinopathy in diabetes and in experimental galactosemia. It is conceivable that hexitol is produced within microvascular cells during periods of elevated blood sugar, and that accumulation of the hexitol results in cell damage and, eventually, retinopathy.

Materials and Methods. The investigations described in this manuscript conform to the ARVO Resolution on the Use of Animals in Research.

Adult dogs were fasted overnight and anesthetized with sodium pentobarbital. Since erythrocytes are known to have hexitol-producing activity, blood cells were removed in situ by perfusion. Heparin was administered, both carotid arteries were cannulated and, after stopping the heart by electroshock, the head was perfused (55 ml/min) with oxygenated Krebs-Ringer bicarbonate containing 4 mM glucose (subsequently called buffer) until perfusate draining from the jugular veins was free of erythrocytes. Eyes and brain were isolated rapidly and placed into cold, oxygenated buffer. Retinas were freed of pigment epithelium, and microvessels were isolated immediately from retinas and cerebral cortex grey matter by a sieving technique. This procedure involves gentle mechanical dissociation of the tissue, followed by retention of the microvessel fragments on a 80-μm nylon sieve. The microvessels were washed with a gentle spray, and if not found to be contaminant-free by phase-contrast microscopy, were again homogenized, sieved, and washed. After isolation, the microvessels were suspended in cold buffer and allowed to settle for 2-3 min. The upper ⅓ of this solution, containing thread-like microvascular fragments, was collected for incubation, whereas the lower ⅔, containing the largest vessels and fragments, was discarded. Since the amount of purified microvessels isolated per retina was extremely small (30-50 μg Lowry protein), fresh retinas from five to seven perfused animals were pooled for each replicate experiment. Lens epithelium, known to have substantial hexitol-producing activity, was studied for comparison. The epithelium together with adherent lens capsule was isolated by stripping it from the anterior surface of the lens.

Samples were divided into two equal portions: one was incubated in 30 mM galactose in buffer (5 hr; 37°C; 95% O₂-5% CO₂), and the other in 30 mM galactose plus Sorbinil (2.5 × 10⁻⁵ M; Pfizer, Inc.). Galactose was chosen as a substrate because its hexitol, galactitol, is further metabolized poorly, thereby providing a good estimate of hexitol-producing activity. After incubation, the tissues were collected on a 20-μm nylon sieve under weak vacuum, washed with 2 ml buffer, and frozen. Internal standard (methyl xylloside) was added, the tissues were disrupted by sonication, and aliquots were taken for protein (Lowry) and polyol analysis. Protein concentration measured after sonication evidently represents cellular protein only (ie, not basement membrane or lens capsule), as sonication reportedly does not disrupt such basement membranes. Samples for polyol analysis were deproteinized (Ba(OH)₂ and ZnSO₄) and
lyophilized, and the polyols were quantitated by gas chromatography of their trimethylsilyl ethers on 3% OV-1. Identification of galactitol was based on its retention time relative to that of the internal standard and was compared with retention times of known polyol standards. Samples were corrected for recovery of internal standard.

**Results.** Microvessels isolated from perfused retina and cerebral cortex showed a normal complement of nuclei, and seemed virtually devoid of nonvascular contaminants, erythrocytes and other nonvascular cells being rarely, if ever, seen (Fig. 1). The majority of the vessels were less than 20 μm in diameter, but a few were as large as 40–70 μm. Electron microscopy revealed that the preparations contained endothelial cells, pericytes, and in the larger vessels, smooth muscle cells. The general architecture of the vessels was preserved, including maintenance of endothelial tight junctions, but some cells were swollen and vacuolated. Tiny glial fragments, not visible by phase microscopy, occasionally were found adhering to the ablumenal side of the vascular basement membrane. These shards generally represented about 1% of the total area occupied by cell cytoplasm in photos of representative capillaries and in no case exceeded 3%.

Galactitol was found to be produced in the retinal and cerebral microvessel preparations (Table 1), although at a rate several-fold less than in the lens epithelium. Sorbinil inhibited hexitol production in all three sites.

**Discussion.** The present study demonstrates the presence of hexitol-producing activity in retinal and cerebral microvessel preparations of dogs. The virtual absence of nonvascular contamination in these preparations leads us to believe that the hexitol-producing activity is present within the vascular cells themselves. It must be acknowledged, however, that the tiny glial fragments found adhering to the microvessels might contribute to this activity. Others have reported polyol-producing activity in microvessels freshly isolated from cows, but the contribution of the vessel cells to this activity is not apparent, since the purity of the vessel preparations is not described, and erythrocytes invariably remain trapped within the isolated vessels unless flushed out.

The identity of the enzyme or enzymes responsible for the hexitol-producing activity in canine microvessels is not clear. Aldose reductase, one enzyme known to have such activity, has not been detected immunohistochemically in retinal or other capillaries of dogs, although immunoreactive aldose reductase has been reported in capillary pericytes of human and monkey retina. Polyol-producing activity has been observed in bovine retinal microvessels incubated in vitro, and the activity has been attributed to aldose reductase on the basis of its greater activity with glyceraldehyde than with glucuronate. This pattern of substrate specificity differentiates aldose reductase from hexonate dehydrogenase but perhaps not from glyceraldehyde dehydrogenase. Glyceraldehyde de-

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<tr>
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<th>μmoles Galactitol/g protein·5 hr</th>
<th>% Inhibition by Sorbinil</th>
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<tr>
<td></td>
<td>Galactose + Sorbinil</td>
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<tr>
<td>Retinal microvessels (n = 5)</td>
<td>24.5 ± 4.1</td>
<td>54</td>
</tr>
<tr>
<td>Cerebral microvessels (n = 6)</td>
<td>13.1 ± 2.7</td>
<td>79</td>
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<tr>
<td>Lens epithelium (n = 20)</td>
<td>167.9 ± 36.8</td>
<td>70</td>
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n = number of replicate experiments; each replicate for retinal microvessel incubations consisted of microvessels pooled from five to seven dogs.
hydrogenase resembles aldose reductase in its ability to reduce glyceraldehyde yet shows no hexitol-producing activity.10

One might speculate that hexitol production plays a role in the pathogenesis of diabetic retinopathy, perhaps via a mechanism analogous to that responsible for the formation of sugar cataract. In the present study, hexitol-producing activity by canine retinal microvessels is found to be several-fold less than that in lens epithelium. It is not known whether this activity is concentrated in a particular cell type in the microvessels or is distributed throughout all the cell types. Endothelial cells cultured from puppy retinal capillaries have been reported to possess hexitol-producing activity.5

Cerebral microvessels, like those from the retina, have hexitol-producing activity, and this activity is inhibited with Sorbinil. This similarity to the retinal microvessels was not to be expected necessarily, since cerebral vessels seemingly are spared many of the lesions characteristic of retinal vessels in diabetes.11 It would be inappropriate to conclude, based on the limited data presented herein, that retinal and cerebral hexitol-producing activities are different.

The presence of hexitol-producing activity within retinal microvessels is consistent with a possible role of hexitol production and accumulation in the etiology of retinal vessel disease in diabetes and experimental galactosemia. Although the hexitol-producing enzyme remains to be identified, its activity, like that of lens aldose reductase, can be inhibited with a known aldose reductase inhibitor. Effects of an aldose reductase inhibitor on the development of retinopathy in diabetic dogs and in galactosemic dogs are under investigation.

Key words: microvessels, polyol, hexose metabolism, diabetic retinopathy, retina

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References


DNA Repair Synthesis in the Rat Retina Following In Vivo Exposure to 300-nm Radiation

Laurence M. Rapp,* Jule Griebrok Jose,f and Donald G. Piris†

Quantitative autoradiography was used to study the incorporation of 3H-thymidine into the retina of albino rats following in vivo exposure to 300-nm radiation. Relative to background labeling in unexposed eyes, there was 8–20 times as much label per unit area in the outer nuclear layer, inner nuclear layer, and ganglion cells of 300-nm exposed retinas. The photoreceptor inner segments also showed thymidine labeling in both control and exposed retinas. Invest Ophthalmol Vis Sci 26:384–388, 1985

Previous studies have shown that DNA repair synthesis occurs in ocular tissues in response to damaging levels of radiant energy. DNA repair in