Effect of Diabetes on Levels and Uptake of Putative Amino Acid Neurotransmitters in Rat Retina and Retinal Pigment Epithelium

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Free amino acid levels and high affinity uptake of glutamate, aspartate, γ-aminobutyrate, glycine and taurine were studied in retina and retinal pigment epithelium of streptozotocin diabetic rats. Results show that experimental diabetes produces a generalized fall in the content of free amino acids in both retina and retinal pigment epithelium. With regard to the high affinity uptake, in the two tissues of diabetic animals showed decreased aspartate uptake, enhanced taurine and γ-aminobutyrate uptake, whereas that of glycine and glutamate was unchanged. These results might suggest that diabetes causes alterations of specific amino acid transport systems and/or alterations of some cell populations.

KEY WORDS: Retina; retinal pigment epithelium; streptozotocin diabetes; free amino acids; uptake.

INTRODUCTION

Diabetes mellitus is known to produce a number of behavioral and pathological abnormalities, including retinopathy. The term diabetic retinopathy usually refers to changes in retinal blood vessels, although other components of the retina may also be affected (1,2). In diabetic patients electroretinographic response and light and color sensitivity are severely reduced in comparison with normal population (3–5). The retinal pigment epithelium (RPE), which is a major transport pathway for the exchange of metabolites and ions between the choroidal blood supply and the neural retina, has been reported to play an important role in a number of ocular lesions, including diabetic retinopathy (2).

In the vertebrate retina a number of studies support a neurotransmitter role of glutamate/aspartate in photoreceptors, bipolar cells and ganglion cells (6), in contrast to the horizontal and amacrine cells, which use the inhibitory neurotransmitters γ-aminobutyrate acid (GABA) or glycine (7,8). Taurine is highly concentrated in retina and RPE, and seems to be required for normal retinal function (9,10). One of the major mechanisms by which amino acid neurotransmitters are inactivated is through a highly affinity, Na+-dependent uptake by neurons and glial cells (11). Therefore, the present work was designed to study the effect of diabetes on the uptake and concentrations of the putative amino acid neurotransmitters L-glutamate, L-aspartate, glycine, GABA and taurine, in the isolated rat retina and RPE.

EXPERIMENTAL PROCEDURE

Animals. Adult Long Evans rats (170–200g) were used in this study. Diabetes was induced by a single intraperitoneal administration of streptozotocin (65 mg/kg) in 0.05 M citrate buffer, pH 4.5 (12). Rats were allowed free access to food and water. The streptozotocin injected animals were used after 20 or 45 days and were considered diabetic if serum glucose exceeded 16 mM.
Fig. 1. Amino acids uptake by normal and diabetic rat retina. Retinas were incubated for 20 min in a Krebs medium containing the radiolabeled amino acid at 20 μM final concentration. Normal retina (□), 20 days diabetic retina (■), 45 days diabetic retina (●). The values are mean ± SEM from eight experiments. * Significant difference from control (p < 0.01).

Fig. 2. Amino acids uptake by normal and diabetic RPE. Incubation was carried out for 20 min in a Krebs medium in the presence of radiolabeled amino acid at 20 μM final concentration. Normal RPE (□), 20 days diabetic RPE (■), 45 days diabetic RPE (●). Results are expressed as the mean ± SEM from five experiments. * Significant difference from control (p < 0.01).

Uptake Experiments. Dark adapted, control and streptozotocin-treated rats were decapitated, the eyes excised, and the sclera was eliminated from the posterior part of the eye. The anterior part of the eye was removed, and the retina and the RPE were gently peeled away using fine forceps. Retina or RPE were incubated under dark at 37°C for 20 min in 1 ml Krebs Ringer medium (118 mM NaCl, 1.2 mM KH2PO4, 4.7 mM KC1, 2.5 mM CaCl2, 1.17 mM MgSO4, 25 mM NaHCO3, 5.6 mM glucose, pH 7.4), containing the radiolabeled amino acid (L-[3H]glutamate, 2.1 TBq/mmol; L-[3H]aspartate, 1.22 TBq/mmol; [3H]GABA, 1.2 TBq/mmol; [3H]glycine, 1.7 TBq/mmol; [3H]taurine, 1.48 TBq/mmol; New England Nuclear, Boston, MA) at a 20 μM final concentration. After incubation, the tissue was washed with cold medium, weighed and dissolved in 0.5 ml of 1% sodium dodecyl sulfate. The accumulated radioactivity was estimated by liquid scintillation counting and concentration of the accumulated amino acid was calculated by its specific radioactivity.

For exchange experiments the tissue was incubated for 20 min in Krebs medium in the presence of non-radioactive amino acid. After that, radiolabeled amino acid was added and the uptake was measured as described in Experimental Procedure. Each value is the mean ± SEM of eight (retina) or five (RPE) experiments.

The extracellular space was estimated as described previously (13). The obtained values were subtracted from amino acids uptake values.

**Table I. Amino Acids Uptake in the Diabetic Rat Retina and RPE**

<table>
<thead>
<tr>
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<th>A-Molar/kg wet weight</th>
<th>B-Molar/kg wet weight</th>
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<tbody>
<tr>
<td>Glutamate</td>
<td>91.3 ± 4.2 79.4 ± 11.5</td>
<td>7.4 ± 0.9 10.4 ± 1.74</td>
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<tr>
<td>Aspartate</td>
<td>39.6 ± 5.7 38.8 ± 9.3</td>
<td>3.2 ± 0.3 5.0 ± 1.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>27.6 ± 1.8 28.4 ± 2.8</td>
<td>5.2 ± 0.4 5.9 ± 0.85</td>
</tr>
<tr>
<td>GABA</td>
<td>105.1 ± 9.0 84.1 ± 15.2</td>
<td>16.1 ± 1.01 16.5 ± 1.47</td>
</tr>
<tr>
<td>Taurine</td>
<td>57.58 ± 5.9 46.5 ± 1.5</td>
<td>11.8 ± 1.04 11.8 ± 1.46</td>
</tr>
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</table>

**A. Uptake:** Tissue was incubated for 20 min in Krebs medium in the presence of the radioactive amino acid (20 μM). B. For exchange experiments, the tissue was incubated for 20 min in Krebs medium in the presence of non-radioactive amino acid. After that, radiolabeled amino acid was added and the uptake was measured as described in Experimental Procedure. Each value is the mean ± SEM of eight (retina) or five (RPE) experiments.

**Amino Acid Concentrations.** Tissues were homogenized in 80% ethanol (1:10 w/v); the protein was sedimented by centrifugation and the supernatant stored at −80°C until use. Amino acids were measured by HPLC after derivatization with o-phthalaldehyde, as previously described (14,15).

Protein content was determined according to Lowry et al (16) using bovine serum albumin as standard. Glucose concentration in serum was determined by the orthotoluidine reaction using a commercial kit (Salubridad, Mexico).

**Statistical Method.** Statistical comparisons were performed with analysis of variance (ANOVA) followed by Tukey's test.

**RESULTS**

**Uptake Studies.** Amino acids uptake by the normal retina and RPE reaches maximum values between 20 and 40 min incubation (not shown, 13,17). Uptake of glutamate, aspartate, glycine, GABA and taurine was highly dependent on extracellular sodium, as previously described (not shown, 13,17,18). Although uptake values of glutamate and glycine were not modified in the retina from diabetic rats, that of GABA and taurine was remarkably increased (50–60%) (Fig. 1). In contrast, the uptake of aspartate was 36% reduced as compared to normal (Fig. 1).

Glutamate, glycine, and GABA uptake in the RPE from diabetic rats did not differ significantly in comparison with normal rats. However, taurine uptake showed a 40% increase and aspartate uptake diminished 48% after 45 days streptozotocin administration (Fig. 2).

We examined the amino acids uptake under conditions where exchange transport was measured. After a preincubation period with non-radioactive amino acid, the uptake of the tritiated amino acid was determined. Under these conditions accumulation values of all the...