The effects of acetazolamide on the human electroretinogram. BIANCA STANESCU AND JEAN MICHELS.

The effects of acetazolamide on the cerebral circulation and metabolism was previously shown to be due to a raised Pco2 in brain tissue. The effects of the drug on the human electroretinogram (ERG) are investigated in fifteen human volunteer subjects. A significant increase in the b-wave was found. A similitude with the mechanism of acetazolamide administration in brain and retinal tissue seems to be a possible explanation.

Up to date, retinal electrogenesis is not definitively explained. The early receptor potential seems to be in relation with photochemistry and the late receptor potential is considered an event at the cellular level. The intracellular recordings showed that the visual cells generate the late receptor potential resembling the a-wave of the electroretinogram (ERG) and the Müller cells generate a late potential similar to the b-wave. However, it is difficult to explain how Müller cells, which are glial cells and without synaptic connections with other retina neurons, are able to reply to photic stimulation. It was suggested that Müller cells, as other glial cells, have a high potential of membrane and therefore are very sensitive to the concentration of K ions. The light-activated neurons in the retina, lead to an increase in external K+ which induce the depolarization of the Müller cell and therefore the apparition of the b-wave on the ERG. The purpose of the present study was to study the alterations of the human ERG following retinal metabolic modifications. To induce them we used Diamox, a carbon anhydrase inhibitor, which produces modifications of the acid-base balance and ion metabolism.

The effect of Diamox (acetazolamide) on the human ERG was not previously investigated.

Materials and methods. Fifteen subjects with an age range between 18 and 58 years were studied. All had a normal ophthalmologic examination (visual acuity, Goldmann perimetry, ophthalmoscopy). We administered Diamox, 500 mg intravenously, in 5 c.c. of physiologic serum to all fifteen subjects.

The control group was formed by ten normal ocular subjects, whose ages ranged between 8 months and 53 years. Five cubic centimeters of physiologic serum was administered to this group intravenously.

Blood samples were taken for ionograms before and five minutes after acetazolamide administration.

The ERG record was performed on a cathode ray storage oscilloscope, Tektronix 5103 N, with a polaroid camera connected to an Ahrend van Cogh electroretinograph and its photostimulator. The recording was performed in "Ganzfeld" with a single-flash stimulation on intensity II of our photostimulator.

The “Ganzfeld” was realized by home-made translucent electrodes. The tests were performed in mesopic conditions (750 Lux). The ERG recordings (six stimulations for each record) were performed 5, 10, and 15 minutes before and after the administration of acetazolamide and physiologic serum.

The mean value of the six stimulations for each record was calculated.

Results. In both groups we found a spontaneous decrease in the amplitude of the b-wave which attained maximum level 15 minutes after the start of the experiment.

However, the mean value of the b-wave in both groups at 15 minutes was not statistically different from the mean value at the beginning (P > 0.10).

A progressive increase of the b-wave amplitude followed the administration of acetazolamide in all subjects investigated. The b-wave started to increase five minutes after injection and increased further five minutes later (Fig. 1).
Reports

Investigative Ophthalmology
December 1975

Diamox

Fig. 1. The amplitude of the b-wave (Mv) (mean value) before and after administration of Diamox (continuous line) and physiologic serum (interrupted line).

Increase of the b-wave 10 minutes after acetazolamide administration was statistically significant (P < 0.001, paired t-test).

There were no changes in the "implicit time" since the intensity of the light stimulation was too high to permit such modifications. There was no consistent change in either sodium or potassium serum levels after Diamox administration.

Discussion. An increase in the carbon dioxide tension (Pco₂) of brain tissue and cerebral blood flow was observed in monkeys, dogs, and man following intravenous administration of acetazolamide. It appears that the drug interferes with carbon dioxide transport by cerebral venous blood, with an accumulation of CO₂ in the brain and, subsequently, an increase in the cerebral blood flow. Previous an increase in the b-wave amplitude was reported in hypercapnia and attributed to a perturbation of the retinal circulation secondary to an increase in blood Pco₂. Administration of vasodilators such as papaverine, priscol, trinitroglycerin, or nicotinic acid results in an increase in the amplitude of the b-wave. Therefore, the increased amplitude of the b-wave which we observed after Diamox administration may be attributed to an increase in the retinal blood flow.

A second mechanism which explains the increase in the b-wave amplitude may be the change in the metabolism of Müller cells following Diamox administration. Indeed glial cells are the main carbon anhydrase-containing cells in the brain. Müller cells are glial cells and therefore one may advance the theory that they are also rich in carbon anhydrase. Kjallquist and Siesjo demonstrated that acetazolamide administration produces a rise in intracellular concentration of H⁺ and HCO₃⁻. More H⁺ is available for the exchange reaction Na⁺-H⁺ and extracellular K⁺ may be in excess, depolarizing Müller cells and increasing the b-wave amplitude. We have no arguments to choose between the two advanced mechanisms.

We are indebted to Prof. M. Meulders, Dean of the Faculty of Medicine, who suggested this study to us.

From the Department of Ophthalmology, Université Catholique de Louvain, Cliniques Universitaires, Service d'Ophthalmologie, Louvain, Belgium. Submitted for publication May 5, 1975. Reprint requests: Dr. B. Stanesco, Université Catholique de Louvain, Cliniques Universitaires, Service d'Ophthalmologie, Capucijnenvoer 7, Louvain 3000, Belgium.

Key words: acetazolamide (Diamox), electroretinogram (ERG), Müller cell, vasodilation, acid-base balance, ion metabolism.

REFERENCES


Crystals in corneal epithelial lesions of tyrosine-fed rats. I. K. GIPSON, R. P. BURNS, AND J. D. WOLFE-LANDE.

Tyrosine-fed rats develop corneal disease which mimics that found in the human metabolic disorder, tyrosinosis. By electron and polarizing microscopy needle-shaped birefringent crystals are demonstrable in early corneal epithelial lesions of tyrosine-fed rats. The crystals appear as negative images by electron microscopy, their content apparently extracted by fixation and/or embedding fluids. Crystals may be arranged in sheaves or bundles and pass from one cell to another disrupting the continuity of membranes of both cells and nuclei. They are present in desquamating epithelial cells along corneal ulcers. Polarizing microscopy of whole mounts of diseased tissue shows that the crystals are limited to epithelial lesion areas. We hypothesize that the crystals are tyrosine and that crystal growth in cells initiates lesion and subsequent ulcer formation.

Corneal ulceration and opacity occur in a rare human metabolic disease, tyrosinosis (Oregon). The disease may also be characterized by chronic dermatitis, elevated serum tyrosine levels, increased urinary excretion of tyrosine and para-hydroxyphenylpyruvate (pHPP), and deficiency of tyrosine aminotransferase (TAT) and/or para-hydroxyphenylpyruvate hydroxylase.

Rats fed a diet containing excess L-tyrosine develop disease which mimics that found in the human disorder. Corneal disease in tyrosine-fed rats begins as bilateral central pinpoint epithelial lesions which develop within 36 to 60 hours after animals are placed on diet. The lesions enlarge, become snowflake in appearance, then confluent. Epithelium may slough to produce an ulcer. Polymorphonuclear leukocytes (PMNL's) infiltrate all layers of the cornea, and stroma edema followed by vascularization occurs. Later (3 to 4 weeks) the epithelium regenerates, vessels shrink, and stroma and endothelium resume a more normal appearance even if high dietary tyrosine is maintained.

This paper reports the presence of needle-shaped birefringent crystals in early lesions. Their damaging effect on epithelial integrity suggests a mechanism for ulcer initiation in this disease.

Materials and methods. Female albino rats (Simonsen Laboratories, Gilroy, Calif.), weighing 140 to 160 gms were fed a potato-base diet supplemented with 5 per cent tyrosine by weight.

Eyes were examined with a Haag-Streit 900 slit-lamp before starting animals on diet. After being placed on diet, experimental animals were examined daily for corneal disease. Animals in all stages (I through VI) of corneal disease were killed for electron and polarized light microscopy.

For electron microscopy enucleated eyes of diseased animals were placed in 5 per cent glutaraldehyde in 0.5 M cacodylate buffer or in 2 per cent glutaraldehyde-1 per cent paraformaldehyde in 0.67 M cacodylate buffer. A 3 mm. trephined central corneal area was then removed and cut in half. Tissue was postfixed in 1 per cent osmium tetroxide in 0.5 per cent cacodylate buffer (pH 7.4) and embedded in Epon-Araldite. Lesions were located by light microscopy in 1 µ-thick sections stained with methylene blue-azure II-basic fuchsin. Tissue blocks were then trimmed, and thin sections of lesions were stained with uranyl and lead salts for electron microscopy.

For polarized light microscopy, fresh or ethanol-fixed 1.5 mm. trephined corneal buttons and epithelial scrapings were examined. Comparisons of tissue crystals and L-tyrosine crystals was carried out using a Zeiss polarizing microscope.

Results.

Electron microscopy. Earliest morphologic evidence of corneal disease occurs in the epithelium within 36 to 60 hours after animals are placed on diet. Stroma and endothelium appear normal. Biomicroscopic pinpoint lesions appear in light microscopy sections as a group of dark-staining cells extending the full thickness of the epithelium (Fig. 1, inset). One or two cells at the basement membrane are involved. Affected cells may be elongated or rounded and vacuoles usually occur in their cytoplasm.

Electron microscopy of these early lesions reveals long slender, spicule- or needle-shaped structures within the affected area (Fig. 1). These structures resemble crystals and appear to be membrane-bound, although continuity of membranes was not always demonstrable. In cross-section the crystalline structures are round or oval and their diameter ranged from 0.5 to 1.1 µ. Longitudinal segments visible in a thin-section measured from 10 to 25 µ. The structures appear as "negative images," that is, they appear vacant, as if their content had been extracted by fixation and/or embedding fluids. They pass from one cell to another, disrupting cell membranes or carrying a cell membrane from one cell into the adjacent cell (Fig. 2). The "needles" disrupt and penetrate nuclei (Fig. 3). Individual needles may be oriented parallel to one another or may splay out in sheaf fashion from a central area (Fig. 1).