CORNEAL LYSOSOMES IN EXPERIMENTAL HERPETIC KERATITIS

B. S. Kasavina, Yu. F. Maichuk,
L. D. Dilgat, and T. V. Ukhina

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Herpetic keratitis is a widespread infectious disease of the eye [1] in which considerable disturbances are observed in the structure of the cornea and the virus penetrates into the deep tissues of the eye [2], with consequent changes in their functional properties. There is evidence in the literature of changes in the activity of lysosomal enzymes in herpetic keratitis [3]. However, the degree of participation of lysosomes in the pathogenesis of this disease has so far been studied extremely inadequately.

The object of this investigation was to study the activity and intracellular distribution of glycosidases (β-glucosidase and β-galactosidase) of the corneal lysosomes and the permeability of their membranes in herpetic keratitis.

EXPERIMENTAL METHOD

The investigation was conducted on 65 sexually mature male Chinchilla rabbits. The animals were divided into two groups: group 1) 20 intact rabbits, group 2) 45 rabbits with experimental herpetic keratitis. A severe form of herpetic keratitis was induced with herpes simplex virus (HSV-1, strain MS, titer of virus 10^{-7} CPD_{50}/0.2 ml), by application of 0.2 ml of virus-containing material to the scarified cornea.

The intracellular localization of glycosidases was judged from the activity of these enzymes in a homogenate and subcellular fractions of the cornea, and permeability of the lysosomal membranes was judged from the ratio of their free activity to total activity, expressed in percent. Triton X-100 was used as detergent to study total enzyme activity. Activity of the glycosidases was determined in homogenates and subcellular fractions (nuclear, mitochondrial, microsomal, and cytosol fraction), by the method of Pattel and Tappel [5]. Protein was

TABLE 1. Lysosomal Glycosidase Activity (in μmoles substrate/min/g protein) in Normal Rabbits and in Rabbits with Experimental Herpetic Keratitis (M ± m)

Enzyme activity	Control	Keratitis			
		3rd day	6th day	10th day	30th day
Cornea					
β-glucosidase					
total	0,7±0,01	0,39±0,06	1,30±0,02	1,32±0,04	1,18±0,06
free	0,32±0,02	0,23±0,01	0,85±0,03	0,92±0,016	0,79±0,04
free/total, percent	42,8±1,6	58,9±1,8	65,3±2,2	69,6±1,3	66,9±1,7
β-galactosidase					
total	0,84±0,02	0,79±0,08	1,28±0,03	1,24±0,03	1,15±0,05
free	0,74±0,03	0,70±0,02	1,27±0,02	1,06±0,03	0,96±0,02
free/total, percent	88,0±1,1	88,6±1,1*	93,7±1,3	85,4±1,4*	83,4±1,2*
Aqueous humor					
β-glucosidase					
total	0	0,53±0,01	0,59±0,005	0,63±0,02	0,62±0,02
free	0	0,40±0,001	0,44±0,007	0,51±0,07	0,47±0,02
free/total, percent	0	75,4±1,2	74,5±1,3	80,9±1,1	75,8±1,3
β-galactosidase					
total	0	0,45±0,09	0,60±0,015	0,64±0,09	0,60±0,01
free	0	0,33±0,09	0,42±0,009	0,52±0,06	0,44±0,01
free/total, percent	0	73,3±1,0	70,0±1,2	83,6±1,3	73,3±1,3

Legend. Mean data of 6 to 8 experiments given ($P < 0.05$); results not differing significantly from the control marked by asterisk.

KEY WORDS: corneal lysosomes; β-glucosidase; β-galactosidase; herpetic keratitis.

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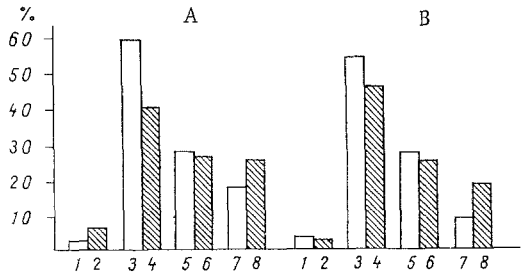

Fig. 1. Distribution of β-glucosidase (A) and β-galactosidase (B) activity in subcellular fractions of cornea in herpetic keratitis. 1, 3, 5, 7) Control; 2, 4, 6, 8) herpetic keratitis. Fractions: 1, 2) nuclear; 3, 4) mitochondrial; 5, 6) microsomal; 7, 8) cytosol. Ordinate, glycosidase activity (in % of total).

determined by Lowry's method [4]. Glycosidase activity in experimental herpetic keratitis was studied during development of the disease and related to its clinical course (on the 3rd, 6th, 10th, and 30th days). To obtain homogenate, 0.25 Msucrose made up in Tris-HCl buffer, pH 7.4, with the addition of 1 mM EDTA, was added to the shredded cornea. The material was homogenized in a homogenizer of Potter type with Teflon pestle and with the PT-2 tissue microblender.

To obtain subcellular fractions the homogenate was centrifuged at 900g for 15 min on the K-24 centrifuge. The residue (nuclear fraction) was washed twice and then resuspended in sucrose. The pooled supernatant fractions were centrifuged at 6000g for 25 min on the VAC-601 centrifuge. The washed residue (fraction of mitochondria) was resuspended in sucrose. The pooled supernatant fractions were centrifuged at 100,000g for 1 h. The supernatant was carefully withdrawn (cytosol fraction) and the washed residue (microsomal fraction) resuspended in sucrose. All operations were conducted at 4°C.

EXPERIMENTAL RESULTS

The results of determination of glycosidase activity and permeability of the corneal lysosomal membranes under normal conditions and in herpetic keratitis are given in Table 1. On the 3rd day of inoculation with the virus into the tissues of the eye a clinical picture of herpetic keratitis developed. The absolute values of total and free β-glucosidase activity were lower than in the control but the percentage of free activity was higher, evidence of a decrease in the stability of the bond between the enzyme and lysosomal membranes. By contrast with β-glucosidase, there was no change in the relative free β-galactosidase activity. On the 6th day after infection of the eye the disease continued to progress. Enzyme activity of the corneal lysosomes was higher than that observed on the 3rd day. The greatest change in β-glucosidase activity was observed on the 10th day of the disease, which corresponded to maximal severity of the inflammatory process in the cornea. By the 30th day of experimental keratitis epithelization of the corneal ulcers and the formation of coarse opacities were observed. Free β-glucosidase activity, expressed as a percentage of the total, remained higher than in the intact rabbits.

On the 3rd day the stability of the bond between enzyme and corneal lysosomal membranes was thus weakened, and this weakening progressed during development of the disease, to reach a maximum on the 6th-10th day. On the basis of these results the localization of glycosidases in cells of the infected cornea was determined on the 6th day after infection. Normally most glycosidase activity (55.4-57.5%) was concentrated in the mitochondrial fraction, in which many secondary lysosomes are known to be sedimented. From 25 to 28% of enzyme activity was localized in the cytosol fraction (Fig. 1). An increase in the outflow of lysosomal glycosidases into the cytosol was observed in experimental herpetic keratitis, parallel with a decrease in their activity in lysosome-containing membrane fractions (mitochondrial and microsomal). This conclusion also was confirmed by the results of experiments to determine lysosomal glycosidase activity in the aqueous humor of the eye (Table 1). The presence of activity of these enzymes in the aqueous humor (normally absent) may be due to their outflow from the cornea into the extracellular space.

It can be concluded from these results that labilization of the lysosomal membranes and weakening of binding of enzymes with them take place as a result of the action of herpes simplex on the cornea, thus contributing to an increase in enzyme–substrate contact and to excessive breakdown of intra- and extracellular glycosaminoglycans. This probably explains the appearance of erosions in the epithelial layer of the cornea, with the formation of extensive ulcers in severe cases, involving the stroma, and giving rise to the subsequent spread of in-

flammation to the uveal tract. The results suggest that corneal lysosomes may participate in the pathogenesis of herpetic keratitis.

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In vitro ACTIVATION OF RAT GASTRIC MUCOSAL ADENYLATE CYCLASE BY TETRAGASTRIN

A. A. Karelin and L. M. Petukhova

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Gastric ulcer is accompanied by hypersecretion of hydrochloric acid and increased adenylate cyclase (AC) activity [3]. Histamine is the principal pharmacological stimulus increasing hydrochloric acid secretion by the stomach [1, 5, 6, 10, 12]. According to the model suggested in [6] histamine is the essential stage in the stimulation of hydrochloric acid production by the stomach. An intermediate link in this chain is 3'5'-AMP. However, some workers were unable to find AC in the gastric mucosa of rats sensitive to histamine [6, 13].

C-terminal tetra- and pentapeptides of gastrin are known to stimulate hydrochloric acid secretion by the stomach in dogs [14]. The presence of gastrin receptors also has been shown in the parietal cells of the stomach [11] and AC has been found in cells of the gastric mucosa sensitive to pentagastrin [4, 9].

The object of this investigation was to test the action of tetragastrin and histamine on AC activity in the rat gastric mucosa and to study the effect of cimetidine (an antagonist of histamine H_2-receptors) on these effects.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 160-180 g. The total number of animals used was 46. Homogenate from the gastric mucosa (100 mg tissue to 1 ml 50 mM Tris-HCl buffer, pH 7.6) was prepared manually in the cold in a glass Potter's homogenizer with Teflon pestle (20 strokes). AC activity of the homogenate from gastric mucosa was determined by ion-exchange chromatography [2]. The main reaction mixture (total volume 60 µl) contained 50 mM Tris-HCl buffer, pH 7.6, 15 mM $MgCl_2$, 45 mM creatine phosphate, 1 mg/ml creatine phosphokinase, 3.0 mM 3',5'-AMP, 0.6 mM ^{3}H-ATP (specific activity 0.1 Ci/mmole), 50-150 µg protein. The biologically active substances to be tested (histamine, tetragastrin, cimetidine) were added to the reaction mixture immediately before the beginning of the reaction. The reaction was started by addition of protein to the reaction mixture and allowed to continue for 10 min at 32°C. The reaction was stopped by diluting the reaction mixture with 0.4 ml of a solution of unlabeled ATP (10 mM) and ^{14}C-3',5'-AMP (10,000 cpm), pH 6.0.

The ^{3}H-3',5'-AMP formed as the result of the reaction catalyzed by adenylate cyclase was separated from other labeled products and ATP by consecutive chromatography of the mixture on columns with Dowex 1 × 8, 100-200 mesh, Al_2O_3, and Dowex 1 × 8, 200-400 mesh. Addition of a known quantity of ^{14}C-3',5'-AMP to the reaction mixture enabled the actual yield of ^{3}H-3',5'-AMP to be determined after chromatography of each sample. Radioactivity was counted on an SL-40 liquid scintillation spectrometer (Intertechnique, France), using the program for double labeling with quenching. The yield of 3',5'-AMP after all stages of chromatography averaged 60-70%.

KEY WORDS: adenylate cyclase; gastric secretion; histamine; tetragastrin; cimetidine.

Laboratory of Biochemistry, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow.

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