EFFECT OF S-ADENOSYL-HOMOCYSTEINE AND DIBUTYRYL-3',5'-AMP ON LYSOSOMAL MEMBRANES IN TISSUES OF THE EYE IN OPHTHALMIC HERPES

B. S. Kasavina, N. A. Fedorov,
Yu. F. Maichuk, and L. D. Dalgat

UDC 617.713-002-022:578.825.11]-092.9-085.
281.8-036.8:617.7-18.11:576.311.342

KEY WORDS: S-adenosyl-homocysteine; dibutyryl-3',5'-AMP; lysosomal membranes; herpetic keratitis.

A number of synthetic analogs of S-adenosyl-homocysteine (SAH), such as 5-deoxy-5-S-isobutyryl adenosine [5], and some of its natural analogs, especially sinefungin [7], are powerful inhibitors of virus replication. There are data in the literature to indicate that cyclic AMP and its analogs not only have an antiproliferative action, but also inhibit the liberation of lysosomal enzymes [2, 3]. It was shown previously that antiviral preparations—iododeoxyuridine, bromouridine, interferon, Prophthalmol — can affect the state of lysosomal membranes of intact eye tissues by changing the strength of the enzyme-membrane bonds or by disturbing the process of lipid peroxidation (LPO) in them [1].

In view of the effects described above it was decided to study the effect of SAH and dibutyryl-3',5'-AMP (DB-AMP) on rabbit eye tissue lysosomes and also to assess their therapeutic properties in herpetic keratitis, by determining the activity of lysosomal enzymes and the malonic dialdehyde (MDA) content.

EXPERIMENTAL METHOD

Experiments were carried out on 57 sexually mature male chinchilla rabbits. The animals were divided into four groups: group 1) 25 intact rabbits, group 2) 12 intact rabbits receiving the preparations, group 3) 12 rabbits with experimental herpetic keratitis treated with the preparations, and group 4) rabbits infected with the virus but untreated.

The preparations were instilled into both eyes of the animals of group 2 in a dose of 0.5 ml to each eye for 10-15 min. The rabbits were killed 1 h after application of the preparations and the eyes were enucleated and the tissues isolated. The preparations were instilled into the eyes of the rabbits of group 3 four times a day. A severe form of ophthalamic herpes was produced by herpes simplex virus (HSV-P, 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 permeability of the lysosomal membranes was judged by the ratio, in per cent, of free enzyme (β-glycosidase and β-galactosidase) activity to total activity, determined in eye tissue homogenates by the method described in [6]. Triton X-100 was used as the detergent to study total enzyme activity. Protein for calculation of enzyme activity was determined by Lowry's method [4]. The course of LPO in the eye tissues and its intensity were judged by the MDA content, determined by the method described in [8].

EXPERIMENTAL RESULTS

On the 3rd day after infection of the rabbits with HSV the animals developed keratitis. In untreated animals with experimental keratitis the course of the disease was much more severe than in the treated rabbits. On the 6th-10th day of infection of the cornea deep ulcers were observed in it, with marked infiltration, iridocyclitis, and vascularization. Epithelization of the cornea ended by the 30th day of the disease with the formation of coarse opacities. Activity of the lysosomal glycosidases of the cornea, iris, and ciliary body in the

Moscow Helmholtz Research Institute of Eye Diseases. Central Research Institute of Hematology and Blood Transfusion, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 27-29, March, 1982. Original article submitted June 19, 1981.

TABLE 1. Effect of SAH and DB-AMP on Activity of Lysosomal Glycosidases in Ophthalmic Herpes (ratio of free to total activity in %; M ± m)

Tissue	β-Glycosidase				β-Galactosidase			
	normal	acute period of ophthalmic herpes	after treatment with SAH	after treatment with DB-AMP	normal	acute period of ophthalmic herpes	after treatment with SAH	after treatment with DB-AMP
Cornea	42,8±2.0	65,3±2,2	39,0±1,2	40,2±1,2	68,0±1,1	93,7±1,3	80,0±1,0	63,4±1,0
Ciliary body	36,0±1,2	68,5±0,9	28,0±1,0	31,0±1,0	67,0±1,3	82,0±1,5	52,4±1,1	59,0±1,1
Iris	43,4±1,1	77,7±0,9	43,0±1,1	38,0±1,0	78,9±1,1	84,4±1,3	65,8±1,2	70,9±1,2
Sclera	44,1±1,3	65,4±1,0	43,5±1,3	57,3±1,2*	91,6±1,4	96,2±1,1*	93,6±1,4	92,9±1,4*
Aqueous humor	0	74,5±1,3	0	0	0	70,0±1,2	0	0

*Results not significant.

Legend. Mean values of results of 6-8 experiments, differing statistically significantly from control, are shown.

TABLE 2. Effect of SAH and DB-AMP on MDA Content in Ophthalamic Herpes (in μmoles/min/g protein; M ± m)

Tissue	Normal	Acute period of ophthalmic herpes	After treatment with SAH	P_{1-2}	After treatment with DB-AMP	P_{1-3}
Cornea	0,71±0,04	4,87±0,04	0,34±0,01	<0,001	0,76±0,03	<0,01
Iris	0,16±0,04	0,45±0,07	0,11±0,01	<0,001	0,18±0,02	<0,05
Sclera	0,55±0,03	1,33±0,03	0,10±0,01	<0,001	0,18±0,01	<0,01
Aqueous humor	0,25±0,02	0,82±0,02	0,13±0,01	<0,001	0.29±0,01	<0,001

Legend. Mean values of results of 6-8 experiments shown.

acute period of the deep form of ophthalmic herpes, and also the ratio of free to total activity were higher than normally (Table 1). Outflow of the glycosidases into the aqueous tumor also was noted. This is evidence of labilization of the lysosomal membranes in the tissue tested, possibly contributing to their destruction.

It was also found that in ophthalmic herpes the intensity of LPO processes in the eye tissues is increased (Table 2), and this may lead to labilization of the cellular and subcellular membranes.

In the study of the effect of SAH and DB-AMP on activity of lysosomal glycosidases and on LPO in intact eye tissues, their optimal dose was determined. A 0.2% solution of DB-AMP had practically no effect on lysosomal membranes of the test tissues. When a 0.3% solution of DB-AMP was used, the lysosomal membranes of the cornea and ciliary body were stabilized. The DMA level fell in the cornea and sclera, indicating depression of peroxidation reactions in these tissues. SAH, in the form of a 0.01% solution, did not change the activity of the lysosomal enzymes, but in the form of a 0.05% solution, it reduced the ratio of free to total glycosidase activity in the cornea, ciliary body, and iris, i.e., it stabilized the lysosomal membranes of these tissues. Enzyme activity did not change significantly in the aqueous humor of the anterior chamber and in the sclera. The MDA level in all tissues and in the aqueous humor of the eye was depressed. Accordingly, a 0.3% solution of DB-AMP and 0.05% solution of SAH were used for the treatment of experimental herpetic keratitis.

The course of the disease in the rabbits treated with the preparations was milder than in the group of untreated animals. For instance, no ulcers were observed in the cornea and the iritis was serous in character. Epithelization of the cornea in animals receiving DB-AMP was complete on the 11th day, whereas in rabbits receiving instillations of SAH it occurred on the 6th day.

Activity of lysosomal glycosidases and the ratio of free to total activity fell in the group of animals receiving DB-AMP practically in all eye tissues studied (Table 1), evidence of stabilization of their lysosomal membranes. This also was confirmed by the absence of enzymes in the aqueous. Normalization of the MDA level also was found in the cornea and aqueous humor of the anterior chamber, and to a lesser degree in the iris (Table 2), evidence of depression of LPO in these tissues.

In the group of animals receiving a 0.05% solution of SAH, free and total activity of the enzymes and also the ratio between them fell in the aqueous humor of the anterior chamber and in all the eye tissues tested except the sclera (Table 1). In these rabbits peroxidation reactions were depressed in all the tissues studied, and by a greater degree than in the animals treated with DB-AMP.

It was thus shown experimentally that DB-AMP and SAH stabilize lysosomal membranes of the eye tissues and exhibit antioxidant properties.

On the basis of the results of these experiments the use of these two substances for the treatment of herpetic keratitis can be regarded as promising.

LITERATURE CITED

1. B. S. Kasavina and Yu. F. Maichuk, Vestn. Oftal'mol., No. 1, 37 (1980).
2. N. A. Fedorov, The Biological and Clinical Action of Cyclic Nucleotides [in Russian], Moscow (1979).
3. L. I. Ignarro and C. Colombo, Science, 180, 1181 (1973).
4. O. H. Lowry, N. J. Rosebrough, et al., J. Biol. Chem., 193, 265 (1951).
5. V. Pattel and A. Tappel, Biochim. Biophys. Acta, 80, 326 (1969).
6. A. Tappel and H. Zalkin, Arch. Biochem., 80, 326 (1959).
7. C. S. Pugh, R. T. Borchardt, and N. O. Shone, J. Biol. Chem., 253, 4075 (1978).
8. M. Gobert-Gero, F. Lawrence, G. Farrugia, et al., Biochem. Biophys. Res. Commun., 65, 1242 (1965).

EFFECTS OF ANTIDEPRESSANTS OF DIFFERENT CHEMICAL STRUCTURE ON UPTAKE AND LIBERATION OF NORADRENALIN BY RAT CORTICAL SLICES

A. T. Dolzhenko

UDC 615.214.32.015.42:612.825:577.175.523

KEY WORDS: antidepressants; [^{14}C]-noradrenalin; uptake; liberation.

The aminopotentiating effect of the antidepressants, first described by Sigg [11], has been associated with their ability to inhibit reassimilation of amines [2], although no parallel has been found between the aminopotentiating action and ability to inhibit uptake in experiments either on nerve–muscle preparations [13] or nerve cells [4]. Meanwhile, when uptake-1 and uptake-2 are blocked, the potentiating effect of melipramine and other antidepressants is preserved [1]. Recently clinically effective antidepressants whose aminopotentiating properties and ability to inhibit neuronal uptake of noradrenalin, serotonin, and dopamine is weak or absent altogether have appeared [2], and the substance mianserin [3] and certain tri- and tetracyclic antidepressants have been shown to be capable of stimulating mediator release from axon endings of noradrenergic neurons.

The object of this investigation was to study relations between the ability of antidepressants with different chemical structure to inhibit reassimilation of noradrenalin and to stimulate its presynaptic release.

EXPERIMENTAL METHOD

Experiments were carried out on slices of rat cerebral cortex prepared by McIlwain's method [8]. The cortex is rich in noradrenalin-containing terminals [6], but at the same time it binds antidepressants with marked affinity [10]. The effect of the antidepressants on noradrenalin release was studied by Farnebo's method [7]. Thin slices (200-250 μ) of rat cerebral hemispheres, incubated beforehand for 30 min with [^{14}C]noradrenalin (1.2×10^{-7} M)

Department of Pharmacology, Donetsk Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR M. D. Mashkovskii.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 29-31, March, 1982. Original article submitted June 9, 1981.

0007-4888/82/9303-0259$07.50