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

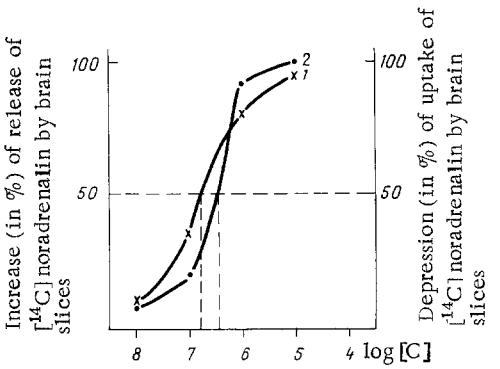

Fig. 1. Effect of imipramine (melipramine) on uptake and release of [^{14}C]noradrenalin by terminals of noradrenergic axons in rat cerebral cortex. Log concentration — effect curves of melipramine obtained by measuring uptake (1) and release (2) of [^{14}C]noradrenalin by cortical slices. Ordinate: left) increase (in %) in release of [^{14}C]noradrenalin by brain slices, on right) inhibition (in %) of uptake of [^{14}C]noradrenalin by brain slices.

in the presence of pargyline (1.25×10^{-4} M), and washed, were stimulated by square pulses (5 Hz, 2 msec, 12 mA) for 2 min. Electrical stimulation of the slices was applied 3 min after the beginning of perfusion, at the time when the outflow of radioactive label had come out on a plateau. The outflow of radioactive label during electrical stimulation was expressed by a release factor [5]. The concentration of antidepressants doubling the outflow of label compared with that in response to electrical stimulation only was determined. Points on the concentration—effect curve were determined by averaging data for 8-12 sections.

In the study of the action of antidepressants on reassimilation the slices were preincubated for 10 min with [^{14}C]noradrenalin (1.2×10^{-7} M) and washed in cold incubation medium, dried, weighed, and solubilized in the presence of 5% Triton X-100 solution. Inhibition of uptake of [^{14}C] noradrenalin by the brain slice tissue was expressed in per cent and determined by the equation in [9]. Activity of the substances was expressed by the value of IC_{50}. The results were subjected to statistical analysis at the P = 0.05 level. Antidepressants with bicyclic (Lu3-010 and Lu5-003), tricyclic (melipramine, amitriptyline, desipramine, nortriptyline, chlorimipramine, noveril, iprindole, C-356), and tetracyclic (pyrazidole, C-394, C-305) structures, and also a polypeptide (thyrotrophin releasing factor — TRF), possessing antidepressant activity, were studied.

EXPERIMENTAL RESULTS

Electrical stimulation significantly increased the outflow of radioactive label from the brain slices (release factor 0.023) compared with the background. In the presence of the various antidepressants studied the outflow of radioactive label from the electrically stimulated brain slices increased even more. The effect of melipramine (Fig. 1) and of the other antidepressants depended on their concentration. The concentrations of the test substances at which release of radioactive label was doubled (EC_{50}) during electrical stimulation of cortical slices are shown in Table 1.

The greatest activity was exhibited by antidepressants with tricyclic structure, headed by amitriptyline ($EC_{50} = 2.4 \pm 0.5 \times 10^{-7}$ M). The weakest activity was shown by antidepressants with bicyclic and tetracyclic structure (EC_{50} for Lu5-003, pyrazidole, and the carbolin derivative C-394 varied from 4.5×10^{-6} to 5.13×10^{-6} M). No dependence of the [^{14}C]noradrenalin-releasing action on chemical structure could be established for the tricyclic antidepressants, although the desmethyl analogs of melipramine and amitriptyline, namely desipramine and nortriptyline, were rather less active.

Melipramine (Fig. 1) and the other antidepressants tested inhibited the uptake of [^{14}C]-noradrenalin by cortical slices independently of their concentration. Values of IC_{50} for these substances varied within wide limits: from $1.05 \pm 0.2 \times 10^{-7}$ M for nortriptyline to

TABLE 1. Effect of Antidepressants on Uptake and Stimulation of Release of [^{14}C]-Noradrenalin by Rat Cortical Slices

Drug	Conc. (in M) doubling release of radioactive label ($EC_{50} \pm S_{\bar{x}} \cdot t$)	Conc. (in M) inhibiting noradrenalin uptake by 50% ($IC_{50} + S_{\bar{x}} \cdot t$)	IC_{50}/EC_{50}
Amitriptyline	$2,4 \pm 0,5 \cdot 10^{-7}$	$5,6 \pm 0,6 \cdot 10^{-7}$	2,3
Melipramine	$2,6 \pm 1,1 \cdot 10^{-7}$	$2,0 \pm 0,3 \cdot 10^{-7}$	0,60
TRF	$3,8 \pm 1,3 \cdot 10^{-7}$	$3,1 \pm 2,2 \cdot 10^{-5}$	81,6
Desipramine	$4,8 \pm 1,6 \cdot 10^{-7}$	$2,1 \pm 0,1 \cdot 10^{-7}$	0,44
Nortriptyline	$5,2 \pm 0,8 \cdot 10^{-7}$	$1,05 \pm 0,2 \cdot 10^{-7}$	0,20
Noveril	$5,75 \pm 3,0 \cdot 10^{-7}$	$7,25 \pm 0,64 \cdot 10^{-6}$	12,6
Iprindole	$5,9 \pm 2,2 \cdot 10^{-7}$	$4,0 \pm 0,9 \cdot 10^{-5}$	68
Chloroimipramine	$6,1 \pm 0,1 \cdot 10^{-7}$	$4,7 \pm 0,3 \cdot 10^{-7}$	0,77
Lu3-010	$3,4 \pm 1,5 \cdot 10^{-6}$	$4,3 \pm 2,3 \cdot 10^{-6}$	1,26
C-395	$3,6 \pm 0,95 \cdot 10^{-6}$	$2,0 \pm 1,2 \cdot 10^{-5}$	5,6
Pyrazidole	$4,5 \pm 1,6 \cdot 10^{-6}$	$6,75 \pm 1,6 \cdot 10^{-6}$	1,5
Lu5-003	$5,13 \pm 0,59 \cdot 10^{-6}$	$5,5 \pm 2,2 \cdot 10^{-6}$	1,1
C-394	$5,13 \pm 0,67 \cdot 10^{-6}$	$6,25 \pm 1,9 \cdot 10^{-6}$	1,2
C-356	$6,21 \pm 0,59 \cdot 10^{-6}$	$4,7 \pm 1,2 \cdot 10^{-5}$	7,6

$4.7 \pm 1.2 \times 10^{-5}$ M for C-356, a tricyclic antidepressant which is a derivative of carbolin. The important point is that for most of the antidepressants tested, concentrations blocking uptake and concentrations potentiating release of [^{14}C] noradrenalin by terminals of cortical noradrenergic axons during electrical stimulation of brain slices do not coincide (Table 1).

As regards substances for which the IC_{50}/EC_{50} ratio is close to unity (melipramine, chlorimipramine, Lu5-003, C-394, Lu3-010, pyrazidole), it cannot be said that they truly possess the ability to potentiate noradrenalin release by terminals of cortical noradrenergic axons, for the increase in radioactivity in the medium during electrical stimulation of the slice may be the result both of increased release of [^{14}C]noradrenalin and of depression of reassimilation of the liberated [^{14}C]noradrenalin. The remaining substances tested can be divided into two groups on the basis of their IC_{50}/EC_{50} ratio: antidepressants for which this ratio is under unity (nortriptyline, desipramine) and antidepressants for which the IC_{50}/EC_{50} ratio is significantly higher than unity (TRF, iprindole, noveril, C-356, C-395, amitriptyline). Evidently the mechanism of the adrenopotentiating action of the latter is due mainly to their ability to stimulate pulsed liberation of the mediator by axon terminals of noradrenergic neurons. Conversely, the adrenopotentiating properties of nortriptyline and desipramine depend on their inhibitory effect on reassimilation of the mediator by terminals of noradrenergic axons in agreement with data on their strong effect on neuronal uptake of noradrenalin [2].

The results are evidence that the mechanism of action of the antidepressants cannot be reduced to their effect exclusively on reassimilation of monoamines by axon terminals of aminergic neurons, The essential mechanism of action of some antidepressants is stimulation of presynaptic liberation of noradrenalin and also, possibly, of other mediators. There are indications that the ability of antidepressants to potentiate noradrenalin release by terminals of noradrenergic axons is due to blocking of presynaptic α_2-adrenoreceptors [3], but these require further investigation, for TRF, which potentiates noradrenalin release by a greater degree than any of the other substances tested (Table 1), evidently has no α-adrenolytic activity.

LITERATURE CITED

1. A. T. Dolzhenko, Byull. Eksp. Biol. Med., No. 5, 556 (1976).
2. M. D. Mashkovskii, A. I. Polezhaeva, and N. I. Andreeva, Khim. Farm. Zh., No. 6, 19 (1979).
3. P. A. Baumann and L. Maitre, Naunyn-Schmiederberg's Arch. Pharmakol., 300, 31 (1977).
4. P. Bevan, C. M. Bradshaw, and E. Szabadi, Br. J. Pharmacol., 53, 459 (1975).
5. A. S. V. Burgen and L. Spero, Br. J. Pharmacol., 34, 99 (1968).

6. A. Dahlstrom and K. Fuxe, Acta Physiol. Scand., 62, 1 (1965).
7. L.-O. Farnebo, Acta Physiol. Scand., Suppl. 371, 19 (1971).
8. H. McIlwain and R. Rodnight, Practical Neurochemistry, Boston (1962).
9. T. Mennini, R. Pataccini, and R. Samanin, Br. J. Pharmacol., 64, 75 (1978).
10. R. Raisman, M. S. Briley, and S. Z. Langer, Eur. J. Pharmacol., 61, 373 (1980).
11. E. B. Sigg, Fed. Proc., 18, 144 (1959).
12. K. Starke, H. Montel, and J. Wagner, Naunyn-Schmiederberg's Arch. Pharmakol., 271, 181 (1971).
13. D. R. Varma and H. M. McCullough, J. Pharmacol. Exp. Ther., 166, 26 (1969).

MICROVISCOSITY OF SUBFRACTIONS OF HIGH-DENSITY LIPOPROTEINS OF HUMAN BLOOD

V. E. Formazyuk, T. I. Torkhovskaya, V. A. Polesskii, A. A. Zadoya*, and Yu. A. Vladimirov

UDC 612.111.7.014.2-06:612.398.145.1

KEY WORDS: lipoproteins; microviscosity; fluorescent probes; hyper-α-lipoproteinemia.

The results of many epidemiologic investigations have shown that a high plasma concentration of cholesterol of high-density lipoproteins (HDLP) is an antirisk factor of ischemic heart disease (IHD) [12]. This fact accounts for the great interest shown by research workers in HDLP and, in particular, in the two subfractions $HDLP_2$ and $HDLP_3$, which differ in composition and properties and which probably perform different functions in the body [6].

The metabolism of the protein–lipid particles of plasma is currently receiving intensive study. One of the essential characteristics of lipoproteins, which determines their role in reactions of this type, is the microviscosity of the lipid regions [7, 10]. Evidence has been obtained of a change in the microviscosity of individual classes of liproproteins in dyslipoproteinemias in man [13], and also in experimental atherosclerosis [3, 5, 9]. Meanwhile the microviscosity of HDLP subfractions in persons with different HDLP cholesterol levels has hardly been studied at all. It has been claimed their antiatherogenic properties are due not only to the high concentration of HDLP, but also to their physicochemical features [4].

The object of this investigation was to study the microviscosity of HDLP subfractions in subjects with different plasma HDLP concentrations. The fluorescent probe pyrene, which has already proved its value in the study of microviscosity of lipoproteins in previous investigations [3, 5], was used to study this problem.

EXPERIMENTAL METHOD

The microviscosity of the principal subfractions of HDLP was studied in 20 subjects aged 30–59 years with different HDLP cholesterol levels (from 33 to 80 mg%). $HDLP_2$ (1.063 g/ml < d < 1.125 g/ml) and $HDLP_3$ (1.125 g/ml < d < 1.210 g/ml) were obtained by consecutive ultracentrifugation [2]. HDLP cholesterol was determined on a Technicon AAII automatic analyzer after preliminary sedimentation of very low- and low-density lipoproteins [8]; phospholipids were determined by Svannborg's method [15].

The microviscosity of the lipoproteins was estimated as the ratio between the intensities of fluorescence at the maximum of the spectrum for two forms of pyrene — the monomer (F_m, 395 nm) and the eximer (F_e, 470 nm) [5]. The fluorescence measurements were made on a

*Deceased.

N. I. Pirogov Second Moscow Medical Institute. All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR E. I. Chazov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 31-33, March, 1982. Original article submitted April 3, 1981.

 0007-4888/82/9303-0262$07.50