LITERATURE CITED

1. R. A. Akhundov, T. A. Voronina, V. V. Rozhanets, and A. V. Val'dman, Byull. Éksp. Biol. Med., No. 3, 329 (1986).
2. T. A. Voronina, Farmakol. Toksikol., No. 6, 680 (1983).
3. M. V. Korablev and P. I. Lukienko, Antihypoxic Agents [in Russian], Minsk (1976).
4. G. N. Kryzhanovskii, A. A. Shandra, and L. S. Godlevskii, Farmakol. Toksikol., No. 6, 13 (1982).
5. R. A. Akhundov, A. V. Val'dman (A. V. Valdman), and T. A. Voronina, Acta Physiol. Bulg., 11, 3 (1985).
6. J. Bureš and O. Burešova, J. Comp. Physiol., 56, 268 (1963).
7. H. Möhler, P. Polc, R. Cumin, et al., Nature, 278, 563 (1979).

ROLE OF DIHYDROPYRIDINE-SENSITIVE Ca CHANNELS IN THE PSYCHOTROPIC EFFECT OF NOOTROPIC DRUGS

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A characteristic sign of aging and age-related memory and learning disturbances is the appearance of changes in the Ca^{2+} regulating system [6, 7]. It has been shown that aging is accompanied by a decrease in the density of voltage-dependent dihydropyridine-sensitive Ca-channels (L-channels) in the cortical association areas of rats [5]. In recent years particular attention has been paid by research workers to the role of Ca channels, and of Ca^{2+} itself, in the processes of memory formation and learning [10], also in connection with the successful practical use of L-channel blockers in various pathological states [4].

In clinical practice, so-called nootropic drugs are being used for the treatment of age-related memory disturbances, and among the best known of them are piracetam and oxiracetam [9]. The mechanism of the pharmacological effect of nootropic drugs has not been completely elucidated, but there is evidence of their involvement in regulation of the cholinergic system [11-13]. In the present investigation we examined the effect of nootropic drugs on brain channels. For this purpose we studied the action of certain L-channel blockers in simple models of memory and learning, in the absence and in the presence of piracetam and oxiracetam. The results were compared with those of the study of the effect of diltiazem, a Ca^{2+}-antagonist, and of nootropic drugs on the concentration of L-channels in rat cerebral cortical membranes.

EXPERIMENTAL METHOD

Blockers of Ca-channels of L type were used: riodipine (Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR), nifedipine, diltiazem (Finland), cinnarizine (Bulgaria), verapamil (USSR), and the nootropic drugs piracetam (Latvbiofarm) and oxiracetam (Research Center for Medical Biotechnology, Ministry of Health of the USSR).

Activity of the drugs was studied on noninbred mice and male Wistar rats weighing 16-20 and 200-220 g respectively, by the method of formation of a passive conditioned avoidance reaction (PCAR), as described previously [2]. The latent period of the first departure from the starting area, and also the number of animals in the group reaching the criterion of training (staying for 5 min on the safe area 24 h after initial training) were recorded.

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TABLE 1. Effect of Ca-Channel Blockers on Recall of Passive Avoidance Reflex Tested 24 h after Training (M ± m)

Preparation	Conditions of administration	Latent period of avoidance reflex, sec		Number of mice reaching criterion of learning after 5 min, %
		initial	after 24 h	
Control		12±2	215±30	50
Foridon	1 h before training	9±1	108±11*	11***
Nifedipine	The same	7±3	163±5	33*
Cinnarizine	» »	7±3	205±6	44
Verapamil	» »	7±2	112±11*	22**
Diltiazen	» »	9±2	107±4**	25**
Diltiazem	Immediately after training	8±3	183±15	40

Legend. *p < 0.05.

TABLE 2. Latent Periods of Avoidance Reaction after Combined and Separate Repeated Doses of Diltiazem and Nootropic Drugs (M ± m)

Preparation	Day of administration				
	0	1st	2nd	3rd	4th
	latent period, sec				
	Mice				
Control	6±1	60±6	109±11	77±8	183±12
Diltiazem	9±3	34±3*	9±3*	16±4*	56±12*
Diltiazem and piracetam	8±1	10±5**	205±11**	119±14**	258±4**
	Rats				
Control	7±3	60±6	121±7	61±3	131±12
Diltiazem	9±4	60±23	15±3*	21±2*	121±8
	Rats				
Diltiazem and oxiracetam	8±2	121±11	200±12**	300±8**	140±21

Legend. Diltiazem was injected 1 h before training or recall, nootropic drugs immediately after training or recall. Piracetam and oxiracetam were injected in a dose of 100, diltiazem – 20 (mice) and 10 mg/kg (rats). *p < 0.05 Compared with control, **p < 0.05 compared with group receiving diltiazem.

The concentration of receptors of 1,4-dihydropyridine (DHP) derivatives in synaptosomal membranes from the rat cerebral cortex was determined with the aid of the labeled probe 2,6-dimethyl-3-methoxycarbonyl-5-([2,3-^{3}H]-p-propoxycarbonyl)-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridine (^{3}H-PMD) (60 Ci/mmole), synthesized in a manner similar to that described previously [1]. Binding of the probe was carried out at 20°C in 20 mM Tris-HCl, pH 7.35, containing 1 mM $CaCl_2$ and 20 mM D-cis-diltiazem. The concentration of membrane protein was 0.1 mg/ml and corresponded to the linear segment of the curve of binding of the probe as a function of protein concentration in the sample. To determine nonspecific binding, a 250-fold molar excess of nitrendipine was added to the samples. Next, 1 h after addition of the probe, 1-ml aliquots were applied to GF/C filters (England) and filtered in vacuo on a water-jet pump, with rinsing twice with 3.5 ml of cold 20 mM Tris-HCl, pH 7.35. The filters were transferred to flasks and radioactivity measured in Bray's scintillation mixture. Protein was determined by Lowry's method, using bovine serum albumin (West Germany) as the standard.

The results were analyzed by the linear regression method and by Student's t-test.

EXPERIMENTAL RESULTS

The ability of Ca-channel blockers of different chemical structure to affect memory formation and the PCAR was estimated in experiments on mice. Intraperitoneal injection of the drugs in a dose of 10 mg/kg 1 h before initial training led to a significant decrease in the latent period of PCAR and in the number of mice in the group reaching the criterion of learning 24 h after the initial training (Table 1). It is interesting to note that of the compounds tested, the maximal and near-maximal effects were given by riodipine, verapamil, and diltiazem, which are derivates of 4-aryl-1,4-dihydropyridine, phenylalkylamine, and thiobenzasenine respectively, bound in different sites on the molecular of the L-type Ca-channel [14]. This fact suggests with a high degree of reliability that the worsening

TABLE 3. Effect of Repeated Injections of Nootropic Drugs on Level of DHP Receptors in Rat Cerebral Cortex (M ± m)

Dose, mg/kg	Days of experiment	B_{max}, fmoles/mg protein	K_d, nM
		Control	
—	—	498±38	1,22(1,56—1,00)
		Piracetam	
100	1	685±57	2,2(2,9—1,8)
	2	788±75	2,3(3,0—1,9)
	3	1037±65	1,9(3,9—1,3)
	4	1090±74	2,3(3,0—1,9)
	5	1073±30	2,1(2,5—1,8)
	6	1075±33	2,1(2,5—1,8)
10	3	1059±43	1,2(1,4—1,0)
1	3	628±41	1,8(2,5—1,4)
0,1	3	426±79	2,0(2,9—1,1)
		Oxiracetam	
100	3	1417±18	1,0(1,5—0,4)*
10	3	1360±72	1,89(2,4—1,6)*
1	3	567±31	1,61(2,0—1,4)*
0,1	3	614±49	1,69(2,6—1,3)*

Legend. Results obtained by analysis of dependence of ^{3}H-PMD binding on ligand concentration in Scatchard coordinates. Asterisk indicates 95% confidence interval.

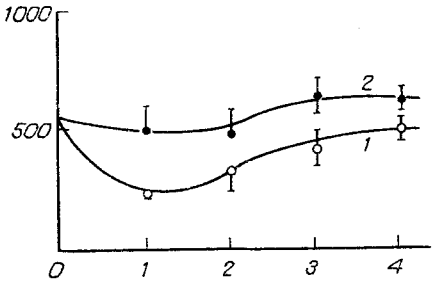

Fig. 1. Time course of change in density of DHP-receptors in rat cerebral cortex after administration of diltiazem (1) and of diltiazem together with oxiracetam (2). Data obtained by analysis of equilibrium binding of ^{3}H-PMD between Scatchard coordinates. Diltiazem and oxiracetam were used in a dose of 10 mg/kg. The animals were decapitated 24 h after the last injection and synaptosomal membranes were isolated. Abscissa, time (in days); ordinate, B_{max} (in fmoles/mg protein).

of recall of the reflex learned in the model, observed following administration of the Ca-channel blockers, is connected with their direct inactivating action on DHP-sensitive Ca-channels. Further confirmation of this hypothesis was obtained when diltiazem was given immediately after training of the mice. Table 1 shows that in this case the blocker had virtually no effect on the parameters measured and, consequently, a memory disturbance is observed only when the initial training is preceded by blockade of Ca-channels.

For a more detailed study of this effect of the Ca-channel blockers of L-type on learning and recall, we studied the effect of repeated injections of diltiazem (10 mg/kg) for 5 days, and measured the latent period of the avoidance reflex 1 h after injection of the blocker (Table 2). Diltiazem is widely used in clinical practice, it is soluble in water, and passes readily through the blood-brain barrier [8]. It was found that just as after a single injection, in these experiments also diltiazem caused a distinct disturbance of recall in the PCAR test in animals of both groups. However, if animals trained after receiving diltiazem were given an intraperitoneal injection of piracetam or oxiracetam immediately after training in a dose of 100 mg/kg, distinct correction of the inhibitory action

of the blocker was observed and in most cases the latent period exceeded that even for the control groups throughout the period of 5 days (Table 2). The results show that piracetam and oxiracetam, which effectively stimulate memory and learning in animals in the model used [2], exhibit marked antagonism relative to the inhibitor action of diltiazem.

To elucidate the possible nature of the observed antagonism between the pharmacological action of diltiazem and the nootropic drugs, we investigated the effect of these compounds on the density of DHP-receptors in the rat cerebral cortex with the aid of the labeled probe ^{3}H-PMD. It is generally accepted that there is a direct connection between DHP receptors and Ca channels of L type, as has been confirmed unambiguously by data on the identification of fully effective Ca channels in artificial membranes containing reconstittued purified DHP receptors [3]. Investigation of binding of ^{3}H-PMD with synaptosomal membranes of the rat cerebral cortex has shown that they contain one class of independent binding sites for the probe with dissociation constant (K_d) of 1-2 nM and with maximal binding capacity (B_{max}) of 0.55 pmole/mg membrane protein. Daily administration of nootropic drugs in a dose of 100 mg/kg had virtually no effect on K_d for binding of the probe, but it led to a marked increase in density of the receptor, up to a maximum on the 3rd day of the experiment, after which it remained unchanged (Table 3). The effect depended on the dose of the preparations, and was maximal with 10 mg/kg. The steady-state level of B_{max} for oxiracetam was significantly higher than that for piracetam, in agreement with the higher pharmacologic activity of the former [2]. The effect of the nootropic drugs on the concentration of DHP-receptors is tissue-specific, for nootropic drugs do not affect binding of ^{3}H-PMD either with membranes isolated from whole brain or with skeletal muscle membranes.

Administration of diltiazem in a dose of 10 mg/kg led to an almost 50% reduction in the density of receptors observed after 24 h, which was followed by gradual restoration of the previous level during subsequent daily injections (FIg. 1). Just as in the case of the nootropic drugs, the value of K_d for binding of ^{3}H-PMD was virtually unchanged. However, simultaneous injection of oxiracetam (10 mg/kg) with diltiazem (10 mg/kg) significantly prevented the decrease in B_{max} and actually raised the level of the receptor on the 3rd and 4th days of the experiment (Fig. 1), in agreement with the results of behavioral experiments.

The results indicate that the antagonism observed in the action of the blockers and nootropic drugs on learning and recall may be mediated through their effect on biosynthesis of Ca-channels of L type. The plasticity of the concentration of channels of this type in the plasma membranes of the cerebral cortex observed in our experiments may perhaps be an important property of the Ca^{2+} regulatory system, determining memory formation and, as a result, the positive effect of nootropic drugs in disturbances of intellectual activity. The results of the present investigation thus also are of fundamental practical importance, in connection with the widespread use of Ca-channel blockers for the treatment of cardiovascular diseases.

LITERATURE CITED

1. V. V. Kastron, I. G. Skrastin'sh, G. J. Dubur, et al., Bioorg. Khim., 15, 256 (1989).
2. V. I. Naumova, I. N. Krylova, Yu. V. Drozd, et al., Byull. Éksp. Biol. Med., No. 6, 711 (1989).
3. V. Flockergi, H. J. Oeken, F. Hofmann, et al., Nature, 323, 66 (1986).
4. J. Glossmann and H. Striessing, Atlas of Science (1988), pp. 1-9.
5. S. Govoni, R. A. Rins, F. Battaini, et al., Brain Res., 333, 374 (1985).
6. Z. S. Khachaturian, Handbook of Studies in Psychiatry and Old Age, ed. by D. W. Ray and G. D. Burrows, New York (1984), pp. 7-30.
7. P. W. Lanfield, F. A. Pitter, and M. D. Applegate, The Hippocampus, ed. by R. L. Issaacson and K. H. Pribram, Vol. 3, New York (1986), pp. 323-366.
8. F. B. Meyer and R. E. Anderson, Mayo Clin. Proc., 61, 239 (1986).
9. B. J. R. Nicolaus, Drug. Dev. Res., 2, 463 (1982).
10. R. A. Nicoll, Science, 241, 545 (1988).
11. F. Pedata, F. Noroni, and G. Pepeu, Clin. Neuro-Pharmacol., 7, Suppl. 1, 272 (1984).
12. H. Pilch and W. E. Müller, Psychopharmacology, 94, 74 (1988).
13. G. Spignoli and D. Pepeu, Eur. J. Pharmacol., 126, 253 (1986).
14. D. J. Triggle, A. Skattebol, D. Rampe, et al., New Insights into Cell and Membrane Transport Processes, ed. by G. Poste and S. T. Crooke, New York (1986), pp. 125-143.