The results of this investigation show that the paraopioid substance dermorphine inhibits DNA synthesis in the corneal epithelium 4 and 24 h after its injection, and also reduces MI 24 h after injection. An increase in MI in the corneal and lingual epithelium took place 4 h after injection of dermorphine. To explain the role of the different subpopulations of opiate receptors in the regulation of cell division further investigations are needed.

LITERATURE CITED

1. A. G. Aleksandrovich, T. F. Zhdanova, S. S. Timoshin, et al., Byull. Éksp. Biol. Med., No. 8, 209 (1989).
2. I. P. Ashmarin and M. A. Kamenskaya, Progress in Science and Technology. Series: Physiology of Man and Animals [in Russian], Vol. 34, Moscow (1988).
3. T. F. Zhdanova and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 9, 354 (1987).
4. T. D. Pan'kova, S. S. Timoshin, M. I. Radivoz, et al., Byull. Éksp. Biol. Med., No. 7, 97 (1988).
5. T. D. Pan'kova and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 7, 96 (1990).
6. P. V. Sergeev and N. D. Shimanovskii, Receptors [in Russian], Moscow (1987).
7. M. I. Titov, V. A. Vinogradov, and Zh. D. Bespalova, Byull. Vses. Kardiol. Nauch. Tsent., No. 2, 72 (1985).
8. E. P. Yarova, "Structural and functional investigation of dermorphine and its analogs," Author's Abstract of Dissertation for the Degree of Candidate of Chemical Sciences, Moscow (1990).
9. S. C. Gilman, J. M. Schwartz, M. J. Milner, et al., Proc. Nat. Acad. Sci. USA, **79**, 4227 (1982).
10. A. A. Smith and N. Wassel, Proc. Inf. Narcotic Res. North Falmouth, Mass., 1979, New York (1980), pp. 255-258.
11. J. Zagon, Brain Res., **412**, No. 1, 68 (1987).
12. J. Zagon and S. R. Goodman, Brain Res., **482**, 297 (1989).

ADRENALIN UPTAKE BY RAT BRAIN SYNAPTOSOMES: EFFECT OF PSYCHOTROPIC DRUGS

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UDC 612.82.018:577.175.522].019.08

KEY WORDS: adrenalin; noradrenalin; uptake; synaptosomes

Investigations have shown that adrenalin (AD) may perhaps play the role of neurotransmitter in the CNS. The following facts may be regarded as proof of this statement: 1) phenylethanolamine-methyltransferase, which synthesizes AD [6], is found in mammalian brain neurons; 2) 6-hydroxydopamine lowers the concentrations of noradrenalin (NA) and AD in the hypothalamus [14]; 3) besides inducing exhaustion of vesicular NA and dopamine (DA), reserpine also reduces the brain AD content [13]; 4) depolarization of neurons in the hypothalamus (potassium, veratridine) leads to release of AD from that structure [5]; 5) a receptor for AD, linked with adenylate cyclase, is found in the brain [15]. Thus AD is synthetized, stored in nerve endings of the brain, and released from them under the influence of depolarization, in agreement with the criteria defining a neurotransmitter. The problem of its inactivation still remains unclear. It can be tentatively suggested that AD, like other monoamines and neurotransmitter amino acids, is inactivated in the synaptic space by reuptake into nerve endings.

All-Union Research Center for Safety of Biologically Active Substances. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 8, pp. 164-166, August, 1991. Original article submitted January 18, 1991.

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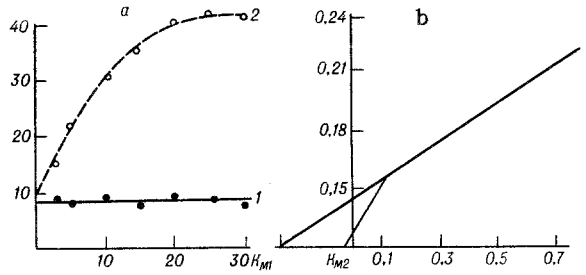

Fig. 1. Uptake of [^{3}H]-D,L-AD by synaptosomes: a) depending on incubation time; abscissa, incubation time (in min); ordinate, uptake of adrenalin (in pmoles/mg protein). 1) Binding of adrenalin by synaptosomes at 0°C; 2) at 37°C. 2 μmoles [^{3}H]-D,L-AD was incubated with 0.2-0.5 mg synaptosomal protein; b) Between Lineweaver—Burk coordinates; abscissa, I [AD] (in μM); ordinate, 1 (in pmoles adrenalin taken up by 1 mg synaptosomal protein in 1 min) (results of 4 or 5 experiments, each with three measurements).

The aim of this investigation was to study AD transport into nerve endings and the effect of psychotropic drugs of different classes on this process.

EXPERIMENTAL METHOD

The coarse synaptosomal fraction (P2) was obtained by centrifugation of a 10% brain homogenate from male albino rats weighing 180-200 g in 0.32 M sucrose at 1000 g for 20 min. The supernatant was recentrifuged at 11,000*g* for 15 min. The resulting residues, containing synaptosomes, mitochondria, and myelin, were resuspended in 0.32 M sucrose.

For determination of dependence of uptake of [^{3}H]-D,L-NA (with specific radioactivity of 30 Ci/mmole, from "Amersham," England) and [^{3}H]-D,L-AD (with specific radioactivity of 3.24 Ci/mmole, from "Izotop," Leningrad) on incubation time and on the mechanism of inhibition of uptake of labeled neurotransmitters by pharmacologic agents, 50 μl of the resulting suspension of the coarse synaptosomal fraction (average 0.5 mg protein) was added to 500 μl of incubation medium containing 100 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 3 mM $MgCl_2$, 10 mM glucose, 100 mM sucrose, 0.54 mM Na_2-EDTA, 0.125 mM pargyline, and 30 mM Tris-HCl buffer, pH 7.4, the labeled mediator, and the drug in the corresponding concentration. The concentration of [^{3}H]-D,L-NA was 1.0 μM, and of [^{3}H]-D,L-AD 2 μM. The samples were incubated for 3 min. Transport of the mediator by synaptosomes was stopped by filtration of 0.2 ml of incubation medium through "Millipore" membrane filters with a diameter of 25 mm (pore diameter 0.45 μ) followed by washing three times with incubation medium (5 ml) at room temperature. Similar parallel experiments were carried out an incubation temperature of 0°C. The filters were dried and dissolved in 10 ml of scintillation fluid containing 7 ml toluene, 3 ml of the methylene ester of ethylene-glycol, 0.5% diphenoxazole (PPO), and 0.01% *bis*-[2-(5-phenoxazolyl)]benzene (POPOP). Radioactivity was measured on a "β-analyzer" (USSR) liquid scintillation counter, and the average number of disintegrations per minute was calculated. Each experiment was carried out in three parallel versions. Protein was determined by Lowry's method [11]. The results were subjected to statistical analysis with calculation of mean values and their confidence limits, at the $p = 0.05$ level.

EXPERIMENTAL RESULTS

Incubation of synaptosomes with [^{3}H]-D,L-AD at 37°C for different time intervals (1-30 min) showed linear accumulation of labeled AD by the synaptosomes during the first 5 min, with flattening out on a plateau after 15-20 min. With an incubation temperature of 0°C, uptake of [^{3}H]-D,L-AD by the coarse synaptosomal fraction was independent of time, and amounted to about 25% of the total uptake (Fig. 1a). The "true" AD uptake by the synaptosomes is the difference between the amount of the monoamine accumulated at 37°C and the amount adsorbed at 0°C. The "true" uptake of

TABLE 1. Constants and Type of Inhibition of Synaptosomal [^{3}H]-D,L-AD and [^{3}H]-D,L-NA Uptake by Monoamines and Some Psychotropic Drugs (M ± m)

Substance	K_i, μm			
	[^{3}H]-D,L-AD		[^{3}H]-D,L-NA	
	K_{M1}= 3,7±0,21 μm	K_{M2} 98,0±47,5 μm	K_{M1} 0,49±0,13 μm	K_{M2} 21,1±7,71 μm
S,L-NA	14,23±2,23	221±79,0	N/D	N/D
L-AD	12,8±1,56	222±86,0	N/D	N/D
D,L-AD	7,1±0,82	N/D	12,0±2,73	104,8±17,4
Dopamine	3,76±0,35	N/D	4,9±0,57	102,8±15,8
Serotonin	1,5±0,52	N/D	0,9±0,3*	15,6±3,2
Pyrazidol	20±4,2	N/D	12,4±4,0	**N/D**
I	152,1±10,3	N/D	135,0±20,3	**N/D**
Viloxazine	37,0±4,0	N/D	150±30*	**N/D**
Zimelidine	64,2±7,2	N/D	71±15*	**N/D**
	43,2±3,3	N/D	67±14*	**N/D**

Legend. *) data from previous publication [1], **) noncompetitive type of inhibition, in all other cases competitive; here and in Table 2, N/D stands for not determined.

[^{3}H]-D,L-AD by the coarse synaptosomal fraction is shown in Fig. 1b in a system of Lineweaver—Burk double reciprocal coordinates. As the graph shows, uptake of [^{3}H]-D,L-AD is reflected in two different kinetic characteristics. Evidently two systems of AD transport function in the coarse synaptosomal fraction: highly specific active transport through the presynaptic membrane against a concentration gradient with high affinity (K_{M1} = 3.7 ± 0.21 μM) and transport with low affinity (K_{M2} = 98.0 ± 47.5 μM). Our results for K_{M1} agree satisfactorily with values of KM obtained on the rat uterus (2.6 μM) and heart (1.4 μM) [7, 9]. Thus the process of accumulation [^{3}H]-D,L-AD by isolated nerve endings depends on the incubation time, the concentration of the monoamine, and the temperature of the incubation medium, it is saturable, and is characterized by the presence of two constants.

There is a close connection between carriers of the monoamines. For instance, the NA-, DA-, and serotonin-transport systems can transport all three mediators [1]. It was therefore important to determine the place of AD in transport systems of the other monoamines. Considering that K_M of uptake in the heart and uterus is nevertheless only about half of the value which we obtained, it can be tentatively suggested that AD has its own carrier in the CNS. To prove this hypothesis an attempt was made to compare AD and NA uptake by rat brain synaptosomes.

The study of the kinetics of synaptosomal uptake of [^{3}H]-D,L-NA revealed the presence of two uptake systems: one with high affinity (K_{M1} = 0.49 ± 0.13 μM, in agreement with data in the literature [1, 8], and a second with low affinity (K_{M2} = 21.1 ± 7.71 μM), which is an order of magnitude less than values of K_M for extraneuronal uptake [8, 12], and five times lower than K_{M2} for AD. The difference between K_{M1} for uptake of AD and K_{M1} for uptake of NA by an order of magnitude may indicate the existence of separate carriers for each of them.

To shed light on the problem of the similarity of AD and NA transport through the synaptic membrane and its stereospecificity, the values of the inhibition constant (K_i) of several monoamines and of some psychotropic drugs on the kinetics of [^{3}H]-D,L-AD uptake by synaptosomes were investigated. Table 1 shows that K_i for [^{3}H]-D,L-AD uptake by L-AD is 3.76 μM, and is only half the value of K_i for D,L-AD (7.1 μM), and coincides with the value of K_M for [^{3}H]-D,L-AD uptake, evidence of the stereochemical specificity of the transport system for AD with higher affinity for L-AD. Stereoisomers of NA competitively inhibit AD uptake systems, but in that case stereospecificity of interaction between the AD carrier and NA molecules could not be found. Meanwhile the value of K_i for high-affinity uptake of [^{3}H]-D,L-NA by L-AD was only half that of K_i by D,L-AD indicating the manifestation of stereospecificity by the NA carrier toward AD isomers. Serotonin and DA also competitively inhibit AD uptake with a value of K_i = 1.5 and 20 μM respectively, indicating that their transport systems differ from those for AD and NA. The antidepressant imipramine noncompetitively inhibits synaptosomal uptake of AD and NA with K_i of 37.0 and 150 μM respectively, further evidence of a difference between AD and NA transport across the synaptosomal membrane. The results confirm our suggestion that AD and NA each has its own carriers in the CNS.

TABLE 2. Effect of Metabolic Inhibitors and Drugs of Different Classes on Synaptosomal Uptake of [^{3}H]-D,L-AD (2 μM) and [^{3}H]-D,L-NA (2 μM) (M ± m)

Substance	Control M	Uptake, per cent of control	
		[^{3}H]-D,L-AD	[^{3}H]-D,L-NA
Control		100±7,0	100±10
Ouabain	$3\cdot10^{-4}$	45,0±3,2	H/O
Protoveratrine A*	10^{-4}	46,4±9,2	H/O
Protoveratrine B*	10^{-4}	51,3±6,6	H/O
2,4-dinitrophenol*	$2\cdot10^{-3}$	57,9±8,1	H/O
Parachloromercuri-benzoate*	10^{-4}	39,8±3,1	H/O
Actinomycin D*	10^{-3}	96,7±1,2*	H/O
Sodium azide	$2\cdot10^{-3}$	41,2±5,9	H/O
Amphetamine	$5\cdot10^{-5}$	21,7±5,2	32±3***
	$5\cdot10^{-4}$	22,4±3,6	27±4***
Sydnocarb	$5\cdot10^{-5}$	46,5±4,7	35,0±4,0
	$5\cdot10^{-4}$	39,3±4,8	33,0±3,0
Chlorpromazine	$5\cdot10^{-5}$	34,7±3,7	28,9±4,1
	$5\cdot10^{-4}$	25,0±5,2	15,3±3,3
Fluphenazine	$5\cdot10^{-5}$	46,3±20,2	39,5±5,6
	$5\cdot10^{-4}$	33,0±18,1	34,1±7,4
Trifluoperazine	$5\cdot10^{-5}$	47,6±6,2	37,5±4,8
	$5\cdot10^{-4}$	33,3±6,7	28,2±2,1
Haloperidol*	$5\cdot10^{-5}$	53,9±5,2	35,2±6,1
	$5\cdot10^{-4}$	40,9±5,0	21,3±7,4
Imipramine	$5\cdot10^{-5}$	46,4±5,9	90,0±10*
	$3\cdot10^{-4}$	44,0±1,9	75,0±6,1
Zimelidine	$5\cdot10^{-5}$	50,4±2,7	67,0±14,0
	$5\cdot10^{-4}$	39,9±2,5	20±3***
Norzimelidine	$5\cdot10^{-5}$	38,7±1,9	56±10***
	$5\cdot10^{-4}$	32,3±5,4	13±3***
Viloxazine	$5\cdot10^{-5}$	60,6±9,8	71,0±15,0
	$5\cdot10^{-4}$	36,1±4,8	18,2±2***
Pyrazidol	$5\cdot10^{-5}$	71,9±3,2	65±7***
	$5\cdot10^{-4}$	43,1±8,1	30±1***
Reserpine*	$5\cdot10^{-5}$	70,8±15,0	35,9±2,1
	$5\cdot10^{-4}$	57,8±8,7	29,1±3,2
Phenazepam*	$5\cdot10^{-5}$	98,1±17,5**	H/O
	$5\cdot10^{-4}$	62,9±6,8	H/O
Diazepam*	$5\cdot10^{-5}$	81,3±8,5**	H/O
	$5\cdot10^{-4}$	78,4±8,1	H/O
Carbamazepine*	$5\cdot10^{-5}$	82,5±18,6**	H/O
	$5\cdot10^{-4}$	74,0±18,2**	H/O
Apomorphine	$5\cdot10^{-5}$	57,2±10,3	H/O
	10^{-4}	41,2±2,8	H/O
Dimethyl sulfoxide (5%)		79,0±6,0	79,0±6,0

Legend. *) substance dissolved in dimethyl sulfoxide (final concentration 5%); **) results do not differ statistically significantly from control at the $p < 0.05$ level; ***) data from previous publication [1].

Investigation of the effect of inhibitors of metabolism on synaptosomal accumulation of [^{3}H]-D,L-AD (Table 2) showed that ouabain, an inhibitor of Na^+,K^+-ATPase, in a concentration of 300 μM inhibits accumulation by 55%. Stabilizers of Na^+-selective ion channels proveratrines A and B (10^{-4} M) also inhibit synaptosomal AD uptake. Uncouplers of oxidative phosphorylation 2,4-dinitrophenol ($2\cdot10^{-3}$ M) and sodium azide ($2\cdot10^{-3}$ M) inhibit uptake of synaptosomal AD by about 50%. The SH-group chelating agent parachloromercuibenzoate (10^{-4} M) also inhibits AD uptake by synaptosomes. Actinomycin D (10^{-3} M), an inhibitor of protein synthesis, does not affect this process.

The results are evidence that the process of AD uptake is stereospecific, is dependent on the presence of energy and a gradient of Na^+ ions, and is independent of protein synthesis.

The results of an investigation of the effect of drugs on labeled AD uptake are given in Table 1. For comparison, data also are given on the action of the same substances on uptake of [^{3}H]-D,L-NA, some of which were published by the writers previously [1]. It will be clear from Table 1 that the strongest inhibitor of uptake of labeled AD and NA is the psychostimulant amphetamine, which in a concentration of 50 μM inhibits uptake of both mediators virtually equally, namely by 70-80%. Sydnocarb inhibits AD uptake rather less strongly than amphetamine.

Neuroleptics of both phenothiazine (chlorpromazine, fluphenazine, trifluoperazine) and butyrophenone series (haloperidol) inhibit NA and AD uptake by a rather lesser degree than the psychostimulants, although there is a tendency for stronger inhibition of NA uptake. These compounds, in a concentration of 500 μM, inhibit uptake of both mediators virtually to the level of sorption.

The antidepressant imipramine, in a concentration of 50 μM, inhibits NA uptake by 10%, which does not differ statistically significantly from the control value ($p = 0.05$), but AD uptake is inhibited by the same concentration by about 50%. The antidepressants zimelidine, norzimelidine, and viloxazine, like imipramine, inhibit AD uptake by a greater degree than NA uptake, whereas pyrazidol, on the contrary, inhibits NA uptake more than AD uptake. The same result also is observed for reserpine. The tranquilizers phenazepam and diazepam, and also the anticonvulsant carbamazepine, in a concentration of 50 μM, had virtually no effect on NA or AD uptake, but in a concentration of 500 μM they inhibited uptake of these mediators by synaptosomes weekly. Apomorphine, a stimulator of dopamine receptors, also proved effective in relation to inhibition of AD uptake.

Thus different classes of psychotropic drugs proved effective inhibitors of synaptosomal AD uptake, possible evidence of their modulating influence of adrenergic transmission in the brain.

LITERATURE CITED

1. E. M. Gankina and N. I. Maisov, Byull. Éksp. Biol. Med., No. 8, 52 (1983).
2. R. N. Glebov and G. N. Kryzhanovskii, Functional Biochemistry of Synapses [in Russian], Moscow (1978).
3. N. I. Maisov, E. M. Gankina, and T. A. Voronina, Farmakol. Toksikol., No. 4, 46 (1985).
4. K. S. Raevskii and N. I. Maisov, Byull. Éksp. Biol. Med., No. 6, 63 (1975).
5. S. K. Burgess and R. E. Tessel, Brain Res., **194**, No. 1, 259 (1980).
6. R. M. Fuller, Ann. Rev. Pharmacol. Toxicol., **22**, 31 (1982).
7. R. P. Green and J. M. Miller, J. Pharmacol. Exp. Ther., **152**, 42 (1966).
8. I. I. Iversen, Brit. J. Pharmacol., **21**, 323 (1963).
9. I. I. Iversen, Brit. J. Pharmacol., **24**, 387 (1965).
10. N. Y. Liang, J. A. Hower, and R. T. Borchardt, Brain Res., **341**, 297 (1975).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. L. Randall, J. Biol. Chem., **193**, 265 (1951).
12. S. R. O'Donnel and N. Saar, Brit. J. Pharmacol., **62**, 235 (1978).
13. B. Scatton and G. Bartholini, Central Adrenaline Neurons: Basic Aspects and Their Role in Cardiovascular Disease, ed. by K. Fuxe et al., New York (1980), pp. 183-193.
14. R. E. Tessel, E. Kennedi, S. K. Burgess, and R. T. Borchardt, Brain Res., **153**, 615 (1973).
15. D. Wilkening, B. Drovkin, M. H. Makman, and J. Y. Lew, Brain Res., **136**, 133 (1980).