LITERATURE CITED

1. E. D. Gol'dberg, A. M. Dygai, and V. I. Agafonov, in: Transplantation of Bone Marrow Under Clinical and Experimental Conditions [in Russian], Moscow (1984), p. 95.
2. E. D. Gol'dberg, A. M. Dygai, and G. V. Karpova, The Role of Lymphocytes in the Regulation of Hematopoiesis [in Russian], Tomsk (1983).
3. P. D. Gorizontov, O. I. Belousova, and M. I. Fedotova, Stress and the Blood System [in Russian], Moscow (1983).
4. A. M. Dygai, Med. Radiol., No. 3, 64 (1984).
5. A. M. Dygai, E. D. Gol'dberg, and L. A. Kolmogorova, Radiobiologiya, 23, No. 3, 349 (1983).
6. B. B. Moroz, V. G. Lebedev, and I. M. Dozmorov, Byull. Eksp. Biol. Med., No. 12, 79 (1983).
7. R. V. Petrov, R. M. Khaitov, V. M. Man'ko, et al., Control and Regulation of the Immune Response [in Russian], Leningrad (1981).
8. S. J. Sharkis, F. Sieber, and L. L. Sensenbrenner, Exp. Hematol., 11, Suppl. 14, 27 (1983).

MECHANISM OF DEPRESSION OF THE SPINAL PAIN SYNDROME BY SEROTONIN DERIVATIVES

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Serotonin (5-hydroxytryptamine, 5-HT) is an inhibitory mediator of the supraspinal descending system [6], and when applied ionotophoretically it inhibits discharges of spinothalamic neurons of the dorsal horn [8] and causes analgesia if applied directly to the spinal cord [16]. 5-HT derivatives may be a promising series of compounds for the relief of pain, but in order to select potentially effective preparations we need to have a deeper understanding of the molecular mechanisms of serotoninergic analgesia. From this point of view there are some interesting data showing that the inhibitory effects of 5-HT on neurons, unlike excitatory effects, are mediated by receptors coupled with adenylate cyclase (ATP-pyrophosphate lyase, cyclizing, EC 4.6.1.1) [13, 15]. On the assumption that receptors of this last type are involved in the antinociceptive action of 5-HT, it was decided to compare the ability of 5-HT derivatives to stimulate adenylate cyclase in the nervous system with their action on the intensity of the spinal pain symdrome (SPS), induced by the creation of a generator of pathologically enhanced excitation (GPEE) in the lumbar segments of the spinal cord [2].

EXPERIMENTAL METHOD

An SPS was induced in rats weighing 200-250 g by applying the sodium salt of benzylpenicillin to the dorsal surface of the lumbar segments of the spinal cord. The intensity of the SPS and its depression by the preparations were assessed according to a 3-point scale [1]. An SPS with a strength of 3 points was characterized by paroxysmal attacks of very severe pain, accompanied by a cry, by motor excitation, flexion of the hind limb, and attempts to bite the skin in an area corresponding to the trigger zone [1]. The preparations were injected intraperitoneally (time of maximal manifestation of the SPS).

The effect of 5-HT derivatives on activity of serotonin-sensitive adenylate cyclase was estimated from the change in cAMP concentration in the synaptosomes, which can be regarded as

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TABLE 1. Effect of Serotonin Derivatives (10^{-3} M) on cAMP Concentration in Synaptosomes

Preparation	Number of experiments	cAMP, pmoles/mg protein	P_t
Control	15	21,8±3,8	
α-5-Hydroxytryptamide of glutamic acid (glumitan)	7	16,0±3,4	
α-Methyltryptamine (indopan)	6	17,1±1,4	
N,N'-dimethyl-5-methoxytryptamine	7	19,0±5,8	
5-(β-Hydroxyethoxy) tryptamine	13	27,2±7,4	
N-acetyl-5-methoxytryptamine (melatonin)	6	33,6±2,8	<0,05
5-Methoxytryptamine (mexamine)	6	61,3±15,4	<0,001
5-hydroxytryptamine (serotonin)	7	65,1±14,0	<0,001
5-Hydroxytryptophan	6	88,0±6,5	<0,001

TABLE 2. Effect of Serotonin Derivatives on cAMP Concentration in Lumbar Enlargement of Spinal Cord

Preparation	Number of animals	Dose, mg/kg	Duration of action, min	cAMP, pmoles/g wet weight of tissue	P_t
Control	15			148±11	
5-Methoxytryptamine (mexamine)	7	5	5	169±12	
	5	5	30	307±49	<0,01
	5	5	60	256±73	
	8	5	120	131±15	
	5	10	90	304±46	<0,01
5-(β-Hydroxyethoxy)tryptamine	5	10	90	201±31	<0,05
5-Hydroxytryptamine (serotonin)	2	10	90	133±21	
N,N'-dimethyl-5-methoxytryptamine	2	10	90	73±16	

Note. Significance of differences compared with control.

miniature anuclear cells, preserving the fundamental metabolic properties of intact neurons [5]. Synaptosomes were isolated from the rat cerebral cortex by the method in [7, 3], and the protein concentration in the suspension of synaptosomes was determined by Lowry's method [11], after which the suspension was diluted so that the protein concentration was 0.2-0.5 mg/ml. To dilute the suspension an incubation medium of the following compositions was used: 125 mM NaCl, 5 mM KH_2PO_4, 1.3 mM $MgCl_2$, 0.77 mM $CaCl_2$, 10 mM glucose, 1 mM theophylline, 20 mM Tris-HCl, pH 7.4. The conditions of incubation of the synaptosomes and of extraction of cAMP were basically the same as in [9]: 1 ml of suspension was incubated at 37°C for 10 min, after which 50 µl of the test preparation was added (final concentration 1 mM), and incubation was continued with continuous shaking for a further 5 min, after which the tubes were placed in an ice bath. Synaptosomes were sedimented by centrifugation with cooling (K-24, East Germany) for 2 min at 12,000 g and the supernatant was removed. The residue of synaptosomes was extracted with 1 ml of 6% TCA, the synaptosomes being thoroughly disintegrated in a homogenizer with glass pestle. The disintegrated synaptosomes were removed by centrifugation (15 min, 12,000 g), after which the cAMP concentration in the supernatant was determined by means of a kit of reagents for radioligand determination of cAMP [12] from Amersham Corporation (England). For this purpose 200 µl of supernatant (acid extract), containing cAMP, was introduced into flasks containining 10 ml of Bray's scintillation mixture and radioactivity was measured on an Intertechnique scintillation spectrometer (France).

The effect of the test compounds on the cAMP concentration in the animals' nerve tissue was tested on healthy noninbred rats weighing 180-200 g. The lumbar enlargement (the region of the spinal cord between lumbar vertebrae L_1-L_4 inclusive) was used because it was in these segments that the GPEE, incuding the SPS, was created experimentally. The wet weight of tissue used for extraction averaged 100 mg. Tissue was taken for investigation at different time intervals after intraperitoneal injection of the preparations, it was homogenized in 3 ml of 6% TCA, the homogenate was centrifuged at 10,000 g for 15 min (K-24, East Germany), and cAMP was determined in the supernatant as described above.

The results were subjected to statistical analysis by Student's test (p_t) and by Wilcoxon's paired test (P_T).

EXPERIMENTAL RESULTS

Values obtained for the cAMP concentration in cerebral cortical synaptosomes (Table 1) coincided with those obtained in [10], when a different method was used for its determination. 5-HT and some of its derivatives tested stimulated cAMP accumulation in the synaptosomes (Table 1). Since the synaptosomes were incubated in the presence of theophylline (a phosphodiesterase inhibitor), the action of 5-HT derivatives on the cAMP concentration could be considered to be the result of adenylate cyclase activation. Maximal activation of cAMP synthesis was induced by 5-hydroxytryptophan (fourfold) and 5-methoxytryptamine (mexamine; about threefold), i.e., the compounds most similar in structure to 5-HT, which also activated adenylate cyclase almost threefold. The effect of 5-(β-hydroxylethoxy)tryptamine was weaker (Table 1), but it must be pointed out that in four experiments this compound almost doubled the cAMP concentration in the synaptosomes compared with the control (41.6 ± 7.3 pmole/mg protein compared with 21.8 ± 3.8 pmole/mg protein; $P < 0.01$). The remaining derivatives, with substituents in both the 5-hydroxyamino group and the amino group, did not affect the cAMP concentration in the synaptosomes.

Considering the powerful stimulating action of mexamine on adenylate cyclase activity in model experiments, we studied its effect (in a dose of 5 mg/kg) on the cAMP concentration in the lumbar enlargement of the spinal cord of healthy animals 5, 30, 60, and 120 min after injection of the compound (Table 2). A twofold increase in the cAMP concentration was found after 30 min under the influence of mexamine. The cAMP concentration in the spinal cord fell to the control level 2 h after injection of mexamine (Table 2). If the dose of mexamine was doubled, a significant increase in the cAMP concentration was observed 1 h after injection of

TABLE 3. Effect of Serotonin Derivatives on the SPS

Preparation	Dose, mg/kg	Number of animals	Depression of SPS, points	Time of action, h	P_T
5-Methoxytryptamine (mexamine)	2,0	4	0,5±0,27*	0,5	
	5,0	13	1,0±0,19*	1,5	<0,01
	10,0	13	1,8±0,18†	2,0	<0,01
5-(β-Hydroxyethoxy) tryptamine	5,0	5	0,7±0,10*	1,0	
	10,0	10	1,5±0,26*	3,0	<0,01
N-acetyl-5-methoxy-tryptamine (melatonin)	5,0	9	—	—	—
	10,0	9	—	—	—
	20,0	8	1,4±0,30	2,5	<0,05
N,N'-dimethyl-5-methoxytryptamine	5,0	7	Changes in behavior	—	—
	10,0	5	The same	—	—

Legend. Intensity of pain syndrome before injection of compound assessed at 3 points. *) Latent period of analgesic effect was about 20 min, †) in three animals the SPS was completely suppressed.

the compound (Table 2). A significant increase in the cAMP concentration at this period also was observed after injection of 5-(β-hydroxyethoxy)tryptamine into the rats. Conversely after injection of the analogous dose of 5-HT and of N,N'-dimethyl-5-methoxytryptamine, no significant changes in the cAMP concentration could be found. The cAMP concentration is shown calculated per wet weight of tissue in Table 2. When calculated per milligram protein of the acid precipitate, the cAMP concentration in the spinal cord tissue (3.7 pmoles/mg protein) was lower than the cAMP concentration in the brain [10].

Table 3 gives data on the effect of 5-HT derivatives on the SPS. Depression of the SPS by mexamine was observed in a dose as low as 2 mg/kg, it increased with an increase in the dose to 5 mg/kg, and in a dose of 10 mg/kg the SPS was completely abolished in three rats and considerably depressed in the majority of animals of this group. A quite strong effect of depression of pathological pain was recorded by the use of 5-(β-hydroxyethoxy)tryptamine, the action of which lasted even longer than that of mexamine. In accordance with the results of experiments on synaptosomes the effect of melatonin on SPS was weak and was exhibited only in a dose of 20 mg/kg. N,N'-dimethyl-5-methoxytrypamine, which did not change adenylate cyclase activity, was completely ineffective against SPS. This compound had an excitatory and psychotropic action on the animals (stereotypy, locomotor excitation, crawling with "shuffling," etc.).

The data described above are evidence of definite correlation between the ability of 5-HT derivatives to stimulate adenylate cyclase *in vivo* and *in vitro*, on the one hand, and their analgesic action on the other hand. Possibly the inhibitory and, in particular, the analgesic effect of 5-HT is the result of a cAMP-mediated increase in permeability of postsynaptic membranes for K^+, which causes the membrane potential to rise [14]. Evidence of the important role of cAMP in the hyperpolarization shift of membrane potential is given by abolition of the hyperpolarization response to injection of 5-HT (for a period of up to 5 h) after injection of selective inhibitors of cAMP-dependent protein kinases into the neuron [4].

LITERATURE CITED

1. E. I. Danilova, V. N. Grafova, and G. N. Kryzhanovskii, Byull. Eksp. Biol. Med., No. 6, 525 (1979).
2. G. N. Kryzhanovskii, Determinant Structures in Pathology of the Nervous System [in Russian], Moscow (1980).
3. V. K. Lutsenko, O. P. Sakharova, and N. G. Lutsenko, Byull. Eksp. Biol. Med., No. 6, 683 (1980).
4. W. B. Adams and I. B. Levitan, Proc. Natl. Akad. Sci. USA, 79, 3877 (1982).
5. H. F. Bradford, in: Handbook of Psychopharmacology, Vol. 1, New York (1975), pp. 191-252.
6. K. Fuxe and G. Johnson, Adv. Biochem. Pharmacol., 10, 1 (1974).
7. F. Hajos, Brain Res., 93, 485 (1975).
8. L. M. Jordan, D. R. Kenshalo, R. F. Martin, et al., Pain, 5, 135 (1978).
9. K. Kobayashi, Y. Kuroda, and M. Yoshioka, J. Neurochem., 36, 86 (1981).
10. L. Lachowitz, R. Woitkowiak, and Y. Ganiszewska, Comp. Biochem. Physiol., C69, 153 (1981).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
12. C. T. Peng, Amersham Radiochemical Centre Rev., 17, 112 (1977).
13. S. J. Peroutka, R. M. Lebovitz, and S. H. Snyder, Science, 212, 827 (1981).
14. M. Segal, in: Neurotransmitter Receptors, London (1980), pp. 89-100.
15. S. H. Snyder, R. F. Bruns, J. W. Daly, and R. B. Innis, Fed. Proc., 40, 142 (1980).
16. T. L. Yaksh, Brain Res., 160, 180 (1979).