ROLE OF GABA-ERGIC AND CHOLINERGIC SYSTEMS IN THE FORMATION OF STATE-DEPENDENT LEARNING INDUCED BY ANTIOXIDANTS OF THE 3-HYDROXYPYRIDINE CLASS

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It was shown previously that membrane-active antioxidants of the 3-hydroxypyridine class, under experimental conditions, possess high psychotropic activity and, in particular, they have a marked anxiolytic and antistressor action [1, 5, 6]. We know that the mechanism of correction of anxiogenic and stressor states by psychotropic drugs is connected with involvement of various receptor and neurotransmitter systems, including catecholaminergic, GABA-ergic, and cholinergic systems. However, the role of neurochemical mechanisms in the realization of the psychotropic effects of 3-hydroxypyridine has not yet been studied.

A convenient approach to the analysis of the action of various substances is to use a model of a dissociated state, when a new functional system is formed against the background of learning and long-term administration of the drug (state-dependent learning – SDL), when conditioned-reflex behavioral responses take place to administration of only that substance, or substances very close to it, against the background of which the response was learned [3, 12, 13]. Under these conditions the use of a substitute test, i.e., replacing the discrimination drug by various pharmacological analyzers, enables neurochemical compensatory changes, indicating the participiation of particular systems in the realization of the action of the drug, to be discovered [3, 4, 9, 11].

In the investigation described below the ability of antioxidants of the 3-hydroxypyridine class to induce SDL was studied and the role of neurotransmitter systems in its formation was analyzed.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 160 g at the beginning of the experiment and 300 g at its end. 2-Ethyl-6-methyl-3-hydroxypyridine (3-HP) was injected intramuscularly in a dose of 150 mg/kg daily for 112 days at 10-11 a.m., and on the day of the experiment, 50 min before testing. The following substances were used as analyzers: bicuculline, a specific blocker of GABA receptors, in a dose of 0.75 mg/kg, subcutaneously 10 min before the experiment, Ca valproate, an inhibitor of GABA-transaminase, in a dose of 200 mg/kg intraperitoneally, 1.5 h before the experiment, Ro-15-1788, a specific blocker of benzodiazepine receptors, in a dose of 15 mg/kg intraperitoneally, 15 min before testing, picrotoxin, a blocker of chloride channels, in a dose of 2 mg/kg intraperitoneally, 30 min before testing, the central cholinolytic benactylzine in a dose of 10 mg/kg intraperitoneally, 1 h before testing, cleregil, a central cholinomimetic, in a dose of 200 mg/kg, 1 h before testing, pyracetam in a dose of 300 mg/kg intraperitoneally, 1 h before testing, the 3-hydroxypyridine analog 3-HP' in a dose of 70 mg/kg intraperitoneally, 1 h before testing, diazepam in a dose of 3 mg/kg interperitoneally, 40 min before testing, ethanol in a dose of 2 ml/kg in the form of a 25% solution intraperitoneally, 20 min before testing, and morphine in a dose of 5 mg/kg intraperitoneally, 20 min before testing. The control animals received injections of physiological saline under the same conditions. Each group consisted of 10 rats. The effect of the substances on the conditioned reflex was studied in a T maze. For this purpose, rats deprived of water for 24 h were placed in the starting compartment

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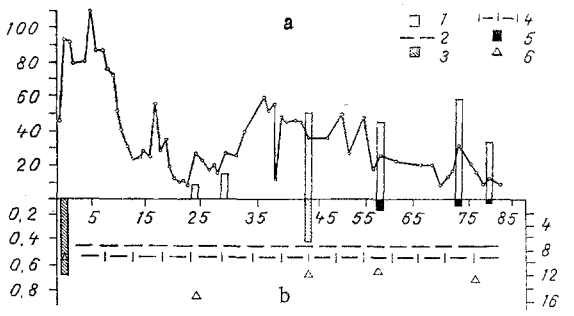

Fig. 1

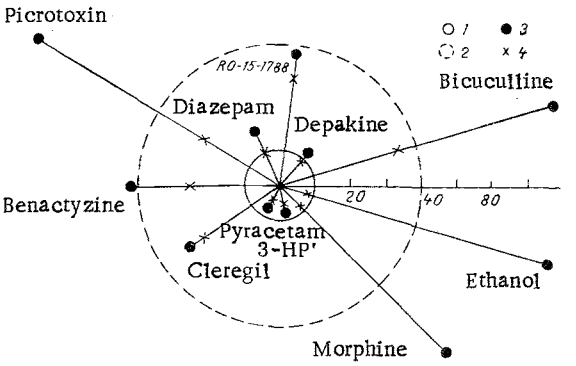

Fig. 2

Fig. 1. Time course of formation of SDL to 3-HP. Abscissa, Time of administration and withholding of drug (in days). a) Effect of 3-HP (150 mg/kg) and its withdrawal of animals' behavior in T maze. Ordinate, average duration of reflex (in sec); b) withdrawal syndrome, expressed as parameters of emotional reactivity and aggressiveness. Ordinate: on left — thresholds of aggressiveness (in μA), on right — emotional reactivity (in points). Parameters of animals' behavior after withdrawal: 1) duration of reflex; 2) control (emotionality); 3) thresholds of aggressiveness; 4) control (aggressiveness); 5) spontaneous aggression; 6) emotionality.

Fig. 2. Analysis of SDL to 3-HP. Duration of reflex in response to: 1) injection of 5-HP; 2) withdrawal of 3-HP; 3) injection of various substances; 4) injection of 3-HP after analyzers.

and, after the conditioned stimulus (a click on opening of the door) the time which the rats spent by the drinking bowl with water, which was located in the right branch of the maze, was recorded. The daily training session consisted of eight trials. The animals were trained after receiving a preliminary injection of 3-HP. The animals' aggressiveness was determined in terms of thresholds of aggressiveness of a pair of rats, induced by painful electrical stimulation through the floor [2]. The emotional characteristics of the animals were estimated on the scale of Brody and Nauta, in the writers' modification [7].

EXPERIMENTAL RESULTS

A single injection of 3-HP had no significant effects on the emotional reactivity or aggressiveness of the animals, but increased somewhat the time of performance of the reflex in the T maze. Conditioning against the background of 3-HP took place in several stages. By the 23rd day of training the rats has reached the criterion of learning, but this was followed by some deterioration, and by the 60th day of injection the animals had reaquired the ability to perform the reflex rapidly. If the drug was discontinued after the 25th, 30th, and 43rd daily dose, the animals' behavior was unchanged, and not until the end of the 2nd month of administration did deprivation of 3-HP induce a disturbance of conditioned-reflex activity, which was expressed as a fourfold increase in the latent period of the reflex (Fig. 1). During long-term administration and discontinuation of 3-HP, against the background of learning, gradual formation of SDL took place, i.e., a system of behavior when the animals were able to perform the conditioned reflex only after prior administration of the drug, and its withdrawal disturbed conditioned-reflex activity. Destabilization of learning between the 23rd and 58th days can evidently be explained by the instability of this system and, together with data showing the slower formation of SDL, they indicate that 3-HP is a substance with low addiction potential.

Parallel with the study of SDL during withdrawal of 3-HP after its long-term administration, the emotional reactivity and aggressiveness of the animals were assessed. By contrast with SDL, these disturbances were formed much more rapidly (Fig. 1). Withdrawal after administration of the drug for 25 days caused a marked increase in emotional reactivity and lowering of the thresholds of aggressiveness. Withdrawal of 3-HP after only 1 week of its administration led to emotional reactivity assessed at 10.9 points on the Brody and Nauta scale, whereas the thresholds of aggressiveness fell to 0.29 μA, and in 50% of the animals signs of spontaneous aggressiveness were observed.

TABLE 1. Effect of Some Drugs on Intensity of Withdrawal Syndrome after Long-Term Administration of 3-HP (M ± m)

Substance	Dose, mg/kg	Emotional reactivity, points	Number of animals with spontaneous aggressiveness, %
3-HP;			
long-term	150	9,2±0,74	30
withdrawal	—	10,9±0,89	80
Benactyzine	10	8,9±1,03	10
Cleregil	200	7,7±1,33	100
Diazepam	3	3,4±0,89	40
3-HP'	70	5,9±1,80	40
Ethanol	2000	3,2±0,89	
Bicuculline	0,75	9,2±0,89	80
Ro-15-1788	10	7,2±0,89	80

The aim of the next series of experiments was to study the role of neurochemical and receptor mechanisms in the realization of action of 3-HP, for which purpose animals with formed SDL to 3-HP were given injections of the various analyzers, as in the replacement test. Considering that the mechanism of action of many psychotropic drugs (tranquilizers sedatives, anticonvulsants, nootropic agents) is to some degree linked with the GABA-benzodiazepine receptor complex and the GABA system, substances modifying the functioning of that system were studied first. Disturbance of behavior in the maze, discovered against the background of SDL to 3-HP, was found to be almost completely abolished by injection of the GABA-positive agent calcium valproate, and it became worse in response to injection of bicuculline and picrotoxin (Fig. 2). By contrast, Ro-15-1788 had no significant effect on the animals' behavior. Analysis of the role of cholinergic mechanisms in the action of 3-HP showed that benactyzine absolished, whereas cleregil potentiated, the animals' aggressive behavior, although neither drug modified DSL (Fig. 2).

To study the specificity of the SDL observed after long-term administration and withdrawal of 3-HP, it was replaced by other psychotropic drugs. It was found that another 3-hydroxypyridine derivative (3-HP') and also diazepam possessed the ability to completely abolish both conditioned-reflex and emotional-behavioral disturbances (Table 1). Pyracetam restored only disturbed conditioned-reflex activity and had no effect on emotional reactivity or aggressiveness of the animals. Ethanol, on the other hand, abolished emotional-behavioral responses and potentiated conditioned reflex disturbances. The action of morphine was characterized by intensification of conditioned-reflex disturbances (Fig. 2).

During long-term administration and withdrawal of 3-HP, during training of rats in a maze, the development of an SDL was thus observed. However, unlike many other psychotropic agents and, in particular, those with high addiction potential (ethanol, morphine), and tranquilizers (phenazepam, chlordiazepoxide) [3, 8, 10, 14], SDL formation against the background of 3-HP takes place slowly, is milder in degree, and is characterized by instability of its parameters, evidence of a fundamental difference in the action of these substances. The formation of a withdrawal syndrome, based on criteria of emotional reactivity and aggressiveness during administration of 3-HP developed much more rapidly, and, evidently, independently of SDL.

The use of the replacement test with analyzers of different neurotransmitter systems showed that they differ in their role in the formation of the withdrawal syndrome. The results are evidence that the principal role in the mechanism of emotional-behavioral disturbances is placed by the cholinergic system, whereas the GABA system is involved in the formation of SDL. The absence of replacement effects in the case of benactyzine and cleregil indicates that the cholinergic system is not a decisive importance in the mechanism of SDL against the background of the antioxidant, and it is evident that more complex mechanisms, conducted with structural and functional changes in the membrane, are involved in the formation of this syndrome.

LITERATURE CITED

1. A. V. Val'dman, T. A. Voronina, L. D. Smirnov, et al., Byull. Éksp. Biol. Med., No. 1, 60 (1985).

2. T. A. Voronina, Yu. I. Vikhlyaev, L. N. Nerobkova, et al., Phenazepam [in Russian], Kiev (1982), pp. 145-151.
3. T. A. Voronina and T. L. Garibova, Byull. Éksp. Biol. Med., No. 1, 49 (1983).
4. T. L. Garibova, K. É. Voronin, and G. M. Rudenko, Byull. Éksp. Biol. Med., No. 11, 570 (1984).
5. K. M. Dyumaev, T. A. Voronina, L. D. Smirnov, et al., Vestn. Akad. Med. Nauk SSSR, No. 11, 3 (1984).
6. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Damage [in Russian], Moscow (1984).
7. T. L. Garibova, V. V. Rozhanets, I. Kh. Rakhmankulova, et al., (Sofia), No. 4, 8 (1985).*
8. E. C. Krimmer and H. Barry, Pharmacol. Biochem. Behav., 13, 313 (1980).
9. H. Lal, G. T. Shearman, S. Fielding, et al., Neuropharmacology, 19, 875 (1980).
10. D. A. Overton, Psychopharmacol. Commun., 2, 339 (1976).
11. D. A. Overton and M. Mayes, Fed. Proc., 39, 402 (1980).
12. D. A. Overton, J. Pharmacol. Exp. Ther., 221, 166 (1982).
13. D. A. Overton, D. A. Mercle, and M. L. Hayes, Anim. Learn. Behav., 11, 295 (1983).
14. I. L. York, J. Psychopharmacol., 74, 339 (1981).

*As in Russian original. Reference incomplete. — Publisher.

ELECTROPHYSIOLOGICAL ANALYSIS OF THE ACTION OF CAVINTON ON SMOOTH MUSCLES

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Cavinton, the ethyl ester of apovincamic acid, is a synthetic derivative of alkaloids of the lesser periwinkle *Vinca minor* and it is used in chemical practice for the treatment of diseases due to cerebrovascular disorders.

The therapeutic action of cavinton is associated with its ability to dilate mainly cerebral vessels. Although there have been many investigations on the clinical aspects of the use of cavinton, the mechanism of its action on smooth muscles has hardly been studied at all [3-7].

The aim of this investigation was a comparative analysis of the action of cavinton on smooth muscles of various organs, using electrophysiological techniques of investigation, whereby changes in membrane potential and membrane conductance of various ions could be monitored, with simultaneous recording of the contractile responses of muscle strips.

EXPERIMENTAL METHOD

The electrophysiological investigations were conducted on spiral muscular strips of bovine cerebral (basilar and posterior communicating) arteries, longitudinal strips from the rabbit portal vein, and the guinea pig taenia coli, by the sucrose gap method, with simultaneous recording of contractions of the muscle strips by means of a mechanotron [1]. The length of the muscle strips was not more than 10 mm and their width 1 mm. The composition of the Krebs' solution was as follows (in mmoles/liter): NaCl, 120.4; KCl, 5.9; $NaHCO_3$, 15.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2, $CaCl_2$, 2.5; glucose 11.5. The cavinton used in the experiments was obtained from Gedeon Richter (Hungary). In the experiments with the hyperpotassium solution, the potassium ion concentration was increased to 80 mmole/liter by the addition of the dry HCl salt to the Krebs' solution. The temperature of the surrounding solution during the tests was 36°C and its pH was 7.4. Electrical and contractile activity of the smooth muscles

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