final stages of whose training took place after administration of the psychostimulants, not only did the number of reflex responses not decrease, but it actually had a tendency to increase: from 80 to 96% in rats receiving the peptide and from 83 to 100% in the rats receiving centrophenoxin.

The results are thus evidence that a single injection of psychostimulants (centrophenoxin or peptide TP-1) is sufficient to bring about statistically significant improvement of the memory trace provided that the substance is given when the consolidation process has already begun. Under these circumstances, the effect of the peptide is comparable in strength with the effect of a typical CNS stimulator.

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COMPARATIVE STUDY OF RELATIVE LIPOPHILICITY OF SOME DIALKYLAMINOALKYL AND DIALKYLAMINOACYL DERIVATIVES OF PHENOTHIAZINE AND THEIR INTERACTION WITH BOVINE STRIATAL DOPAMINE RECEPTORS

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KEY WORDS: dialkylaminoacyl derivatives of phenothiazine; lipophilicity; dopamine receptors.

The difference between the chemical structure of dialkylaminoacyl (DAC) derivatives of phenothiazine and that of typical neuroleptics is replacement of the dialkylaminoalkyl (DAL) group attached to the cyclic nitrogen atom in position 10 of the tricyclic phenothiazine nucleus by DAC [2]. This type of modification leads to a significant change in the pharmacological properties of these compounds. For instance, neuroleptic activity characteristic of DAL representatives disappears or is considerably reduced in DAC derivatives and, at the same time, a number of cardiotropic and, in particularly, antiarrhythmic properties appear [2]. Differences in the pharmacological activity of these two groups of compounds may be associated primarily with the change in their physicochemical properties, which determine the action of the drug at all the principal stages of its existence in the organism [3, 4]. For instance, physicochemical properties play an important role in interaction of the drug with different receptor systems [3, 7]. The principal physicochemical characteristics of a compound which determines its biological activity are its relative hydrophobicity or lipophilicity (LP) and its donor-acceptor and steric properties [9].

The aim of the present investigation was to compare LP and donor-acceptor properties of DAC and DAL derivatives of phenothiazine and also their effect on specific binding of ^{3}H-

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spiperone with bovine striatal type D_2 dopamine (DA) receptors.

EXPERIMENTAL METHOD

Membranes were isolated from the bovine corpus striatum by the method in [10] and kept at the temperature of liquid nitrogen. Binding of ^{3}H-spiperone with striatal D_2 receptors was measured by the method in [10]. The protein concentration was measured by the method in [12]. Distribution of the compound in an octanol—water system was determined as in [9] and in a Ficoli—dextran system as in [5]. The dissociation constant of the compounds was determined by potentiometric titration [14].

The hydrochlorides of chlopromazine, trifluoperazine, and fluphenazine were used. The corresponding hydrochlorides of the DAC analogs of the neuroleptics (compounds G-512, G-219, and G-229) were synthesized in the Laboratory of Mediator Chemistry, Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, by A. N. Gritsenko.

Reagents: monosubstituted and disubstituted sodium phosphate, Tris, and bovine serum albumin were from Sigma (USA); Ficoll (batch 11069) and dextran (batch 580870) from Pharmacia Fine Chemicals (Sweden); octanol was of Soviet origin (chemically pure); ^{3}H-spiperone (35.9 Ci/mmole) was from Amersham International (England).

EXPERIMENTAL RESULTS

To estimate LP of chemical compounds methods based on distribution of the substances in different biphasic systems, based on measurement of the comparative solubility of the distributed substances in the phases of the system [9] are widely used. The equilibrium partition coefficient characterizes the free energy of transfer of substances from one phase into the other [9].

Comparative analysis of the partition coefficients of representatives of the two groups of phenothiazine derivatives studied showed (Table 1) that DAC derivatives are less lipophilic compounds than the corresponding DAL analogs (neuroleptics). These results were obtained when partition coefficients were determined in an aqueous-organic system of octanol-water (log p), the method traditionally used for these purposes [9, 11, 14], and also in an aqueous-polymer system of Ficoll—dextran (ln K), which is the optimal method at the present time for estimation of LP [15]. Values of log P of the hydrochlorides of the DAL derivatives used in the investigation agree with data in the literature [9, 11].

It might be supposed that the absence of neuroleptic activity in DAC derivatives may be connected with their much less ability to pass through the blood-brain barrier and/or with a change in their affinity for type D_2 DA receptors. The view is quite widely held in the literature, based on the study of BEB permeability for many classes of compounds (certain hypnotics and antihypertensives), that LP is one of the important criteria determining the ability of a compound to penetrate into the CNS [3, 4, 14]. For this possibility to be realized the value of log P must be 2 or more [4, 14]. Considering that DAL derivatives of phenothiazine have marked ability to pass through the BEB [3], it can be postulated that the DAC analogs will also possess this property. The values of log P of these compounds exceed the value indicated above (Table 1), for we know [9] that hydrochlorides of the corresponding compounds themselves. The ability of DAC derivatives to penetrate into the CNS also is demonstrated by the fact that they have various central effects [1].

A comparative study of the donor-acceptor properties of the DAC and DAL derivatives showed (Table 1) that molecules of pairs of the corresponding derivatives at physiological pH values virtually do not differ in their degree of ionization. This conclusion can be drawn by comparing values of pK_1 and pK_2 of the corresponding DAC and DAL analogs. The value of pK is known to exert a significant influence on the pharmacological activity of a preparation [8, 11, 14] and, in particular, by its influence of LP of the compound [9]. It is clear from Table 1 that the effect of the degree of ionization of molecules of DAC and DAL derivatives on their LP is virtually identical.

Thus DAC derivatives do not differ in molecular charge at physiological pH values from the corresponding neuroleptics, but they are less lipophilic compounds. This may lead to change in the effect of DAC agents on several mechanisms of cellular regulation, including interaction with receptors [3, 7], with membrane structures [3], with calmodulin [11], etc.

It was accordingly decided to study the action of DAC and DAL derivatives on binding of ^{3}H-spiperone with bovine striatal type D_2 receptors. DAC derivatives were found to be

TABLE 1. Partition Coefficients and Dissociation Constants of Hydrochlorides of Phenothiazine Derivatives (M ± m)

Hydrochlorides of	lg P	ln K	p K_1	p K_2
Chlorpromazine	2,2±0,04 (4)	0,17±0,10 (6)	10,1±0,05 (4)	—
G-512	1,5±0,04 (5)	0,10±0,02 (5)	9,9±0,04 (4)	—
Trifluoperazine	2,6±0,07 (5)	0,18±0,07 (5)	9,0±0,04 (5)	5,3±0,03 (4)
G/219	1,7±0,08 (6)	0,11±0,04 (6)	9,3±0,05 (4)	5,6±0,03 (4)
Fluphenazine	2,5±0,07 (5)	—	8,9±0.03 (5)	5,2±0,03 (4)
G-229	1,8±0,03 (4)	—	8,9±0,04 (5)	5,3±0,03 (4)

Legend. P) Partition coefficient in octanol–water system; K) partition coefficient in Ficoll–dextran system; pK_1) dissociation constant of terminal amino group; pK_2) dissociation constant of amino agroup of piperazinyl radical. Number of experiments shown in parentheses.

TABLE 2. Inhibition of Specific Binding of ^{3}H-Spiperone with Bovine Striatal DA Receptors by Phenothiazine Derivatives (M ± m)

Hydrochlorides of	K_i
Chlorpromazine	10,0±0,80 нМ (4)
G-512	5,0±0,04 мкМ (5)
Trifluoperazine	6,0±0,05 нМ (4)
G-219	10,0±0,10 мкМ (4)
Fluphenazine	1,5±0,10 нМ (5)
G-229	4,0±0,03 мкМ (4)

Legend. K_i) Inhibition constant of specific binding of ^{3}H-spiperone. Number of experiments shown in parentheses.

much less able (by 2-3 orders of magnitude) to displace the radioligand from the receptor (Table 2). This fact may explain the absence or considerable weakening of the neuroleptic effect of DAC agents. Neuroleptics are known to act through their antagonism with central type D_2 receptors [13]. DA receptors also participate in regulation of the circulation, by mediating mainly vasodilatation and bradycardia [5, 6]. The difference in the cardiovascular effects of DAC and DAL derivatives may therefore be partially connected with their different ability to antagonize DA receptor function.

Ability to inhibit binding of ^{3}H-spiperone with type D_2 striatal receptors is therefore much weaker in DAC derivatives than in the corresponding neuroleptics, with the result that their neuroleptic activity is absent or considerably weakened. One cause of the change in affinity for type D_2 receptors may be reduction of the lipophilicity of DAC derivatives of phenothiazine.

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PHARMACOKINETICS, BEHAVIORAL, AND NEUROPHYSIOLOGICAL ASPECTS OF THE ACTION OF 2-ETHYL-6-METHYL-3-HYDROXYPYRIDINE IN RATS

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KEY WORDS: nootropic drugs; pharmacokinetics; behavior; rat.

Derivatives of 3-hydroxypyridine possessed marked psychotropic activity, with the following effects in their spectrum of action: anxiolytic, antistressor, antiamnesic, antianoxic, and anticonvulsant, and in high doses — muscle-relaxant and sedative [2, 5, 6].

The aim of this investigation was to study correlation between the concentration of one of the principal 3-hydroxypyridine derivatives, namely 2-ethyl-6-methyl-3-hydroxypyridine (3-HP), in biological material from animals and the time course of the change in behavioral responses and brain electrical activity of rats.

EXPERIMENTAL METHOD

The pharmacokinetic, behavioral, and electrophysiological investigations were conducted on 143 noninbred male albino rats weighing 180-250 g. The animals were given a single intraperitoneal injection of 3-HP in doses of 50-200 mg/kg. Quantitative analysis of 3-HP in the biological material was undertaken by high-performance liquid chromatography. The method was described in detail previously [7]. The pharmacokinetic parameters were calculated by the use of a one-compartment model with absorption [10]. The mean retention time of 3-HP in the animals was calculated by the statistical moments method [8]. Correlations were calculated by regression analysis.

The tranquilizing effect of 3-HP in a dose of 200 mg/kg was assessed by a conflict situation method, the basic model for evaluation of typical and atypical anxiolytics [11]. The number of punishable takings of water and the number of approaches to the feeding bowl were taken into account. Neurophysiological investigations of 3-HP is a dose of 50 mg/kg were undertaken as unrestrained rats, into which monopolar recording electrodes were implanted by a stereotaxic method 5-6 days before the experiments, into the sensomotor cortex and dorsal hippocampus. Fourier spectral analysis of the EEG of the brain structures of the conscious animals was carried out and changes in potentials with time were analyzed by means of a computerized Berg-Fourier analyzer (O. T. E. Biomedica, Italy).

EXPERIMENTAL RESULTS

The pharmacokinetic investigations showed that in a dose of 150 mg/kg 3-HP could be detected in the plasma, liver, and brain of the animals for 24 h (Fig. 1). Data on the prin-

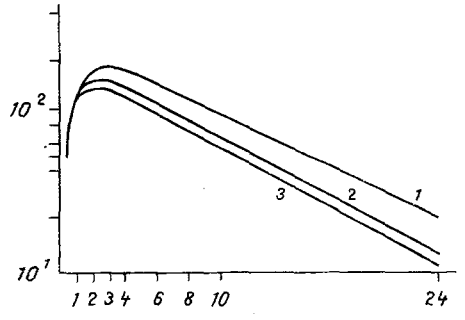

Fig. 1. Kinetic curves of 3-HP concentration in blood plasma (1), liver (2), and brain (3) after intraperitoneal injection in a dose of 150 mg/kg. Absicssa, T, h; ordinate, C, ng/ml, ng/g.

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