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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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UDC 615.214.22.015.4:612.822.3

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-

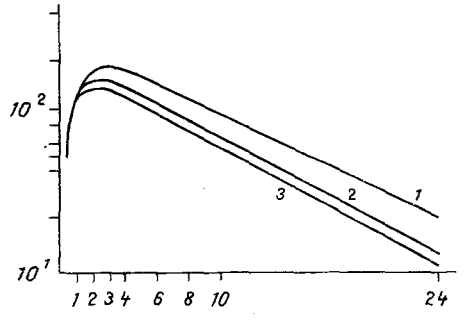

Fig. 1. Kinetic curves of 3-HP concentration in blood plasma (1), liver (2), and brain (3) after intraperitoneal injection in a dose of 150 mg/kg. Absicssa, T, h; ordinate, C, ng/ml, ng/g.

Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR, A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 8, pp. 165-167, August, 1988. Original article submitted May 27, 1987.

 0007-4888/88/0008-1104$12.50

TABLE 1. Pharmacokinetic Parameters of 3-HP in Rats

Parameter	Plasma	Liver	Brain
K_a, h^{-1}	0,74	1,04	0,69
$T_{1/2a}$, h^{-1}	0,94	0,66	1,01
K_a, h^{-1}	0,11	0,13	0,24
$T_{1/2e}$, h^{-1}	6,38	5,33	2,86
V, liters/kg	533	653	582
T_{max}, h	3,0	2,3	2,3
C_{max}, ng/ml	202	171	146
Cl_p, liters/h	57,8	84,9	141,0
MRT, h	10,56	8,73	6,23
AVC, mg·h/ml·kg	2594	1767	1061

cipal pharmacokinetic parameters of 3-HT given in Table 1 show that the substance is absorbed from the peritoneal cavity quite quickly, with a half-absorption time of 0.94 h, and the maximal concentration in the plasma is reached after 3 h and in the liver and brain after 2.3 h. Comparison of the half-elimination time of 3-HP from different biological substrates shows that the substance is excreted most rapidly from the rat brain. Further confirmation of the more rapid elimination of 3-HP from the rat brain also is given by the low value of MRT (6.23 h) and the high clearance (141 liters/h) of the substance by comparison with the corresponding pharmacokinetic parameters calculated for the animals' liver and plasma.

The following changes in the power spectra of the EEG of the brain structures took place after the action of 3-HP in a dose of 50 mg/kg: a significant increase in power of the dominant peak in the 4-8 Hz range (θ-band) in the cortex on average by 48.0 ± 17.4% (maximal effect after 2.3 ± 0.3 h), and in the hippocampus by 6.20 ± 14.7% (maximal effect after 2.0 ± 0.4 h). At the same time there was a tendency for the absolute power of the slow-wave part of the EEG spectrum in the δ-band (0-4 Hz) to decrease, an increase in the absolute power of the α-band (8-13 Hz), and a significant increase in the absolute power of the β_1-frequency band (13-20 Hz). The changes observed in the EEG power spectra (except the increase in the β_1-band) under the influence of 3-HP are close to the changes in structure of the Fourier EEG spectra under the influence of nootropic drugs, namely pyracetam and pyritinol [3-5].

The time course of the change in power of the dominant peak of the cortical and hippocampal EEG spectra is shown in Fig. 2, from which it is clear that there was a gradual significant increase in this parameter, to reach a maximum after 2 h, after which the dominant power gradually diminishes in value. Comparison of the 3-HP concentrations in the plasma and brain of animals with changes in the power of the EEG spectra of the brain structures at corresponding time intervals showed significant high correlation between the power of the dominant peak of the cortical EEG spectrum (X) and the concentration of the compound in the plasma (Y; $Y = 4.32 X + 16.96$; $r = 0.84$; $n = 6$; $p < 0.05$). Similar correlation ($r = 0.88$) was found between the power of the dominant peak (X) and the concentration of the compound in the brain (Y); $Y = 2.98 \pm 28.26$; $r = 0.88$; $n = 6$; $p < 0.05$). Meanwhile no significant correlations were found between the 3-HP concentration in the plasma and brain of animals with changes in the power spectra of the EEG of the dorsal hippocampus.

To discover if correlation existed between the 3-HP concentration in the plasma and brain of the rats and its anxiolytic effect, a series of experiments was carried out to assess the time course of changes in the animals' behavior in a conflict situation at different times after injection of 3-HP. Analysis of the main parameter of the anxiolytic effect, the number of punishable responses (Fig. 3), shows that 10 min after injection of 3-HP (200 mg/kg) no effect was observed, but 30 min later the number of responses was 3 times greater than in the control, and 4.4 times greater than after 60 min. The maximal effect was observed after 2 h, it was reduced after 4-8 h, and completely absent after 24 h.

Analysis of the time course of another parameter of animal behavior in a conflict situation, namely goal-directed motivated motor activity (the number of visits to the feeding bowl) revealed no clear rules governing the dependence of this form of behavior on the time the effect was recorded (Fig. 3). This parameter was the same for many hours after injection of 3-HP.

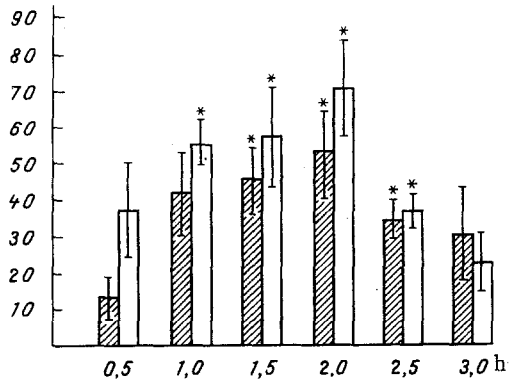

Fig. 2

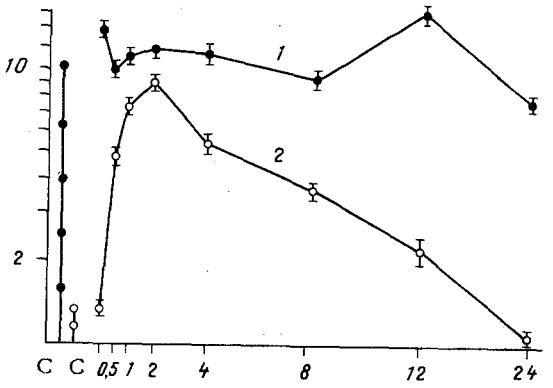

Fig. 3

Fig. 2. Changes (in %) in amplitude of dominant peak of power spectrum of EEG of sensomotor cortex (obliquely shaded columns) and dorsal hippocampus (unshaded columns) of rats with time after injection of 3-HP in a dose of 50 mg/kg. *$p < 0.05$.

Fig. 3. Anxiolytic effect of 3-HP (ordinate), observed in a conflict situation after intraperitoneal injection in a dose of 200 mg/kg. C) Control; 1) number of visits to feeding bowl; 2) number of punishable responses.

Comparison of the results of the study of the time course of the anxiolytic effect (the number of punishable responses) of 3-HP with the time course of its plasma and brain concentrations in the rats revealed significant high correlation. In this case correlation between the 3-HP concentration in the brain and its anxiolytic action was higher ($r = 0.92$) than when the effect was compared with the time course of the plasma 3-HP concentration ($r = 0.83$). These data emphasize the significantly close correlation between the time course of the 3-HP concentration in the brain and the manifestation of its anxiolytic effect.

As a result of this investigation correlation was thus found between the pharmacokinetic parameters and the principal manifestations of the psychotropic effect of 3-HP. Correlations were found between the intensity of the anxiolytic effect and its development in time and the time course of the 3-HP concentration in the rats' brain. It was also shown that the electroencephalographic manifestations of the nootropic effect in the cerebral cortex correlate well with the 3-HP concentration; this confirms the view that nootropic drugs act mainly on the cerebral cortex [12].

As we know, 3-HP is a drug with a marked membranotropic action, capable of modifying the physicochemical properties and structural and functional state of the membranes, the composition of the phospholipids, and the lipid/protein ratio [1, 6, 9]. A high degree of binding of 3-HP with membranes of the endoplasmic reticulum also has been demonstrated in the rat brain [7].

The molecular and pharmacokinetic features of the action of 3-HP on the membrane mentioned above suggest that membrane mechanisms may be involved in the realization of the psychotropic effects of 3-HP, and this may evidently determine the particular features of the pharmacokinetics of this substance.

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EFFECT OF L-DIHYROXYPHENYLALANINE IN BEHAVIOR OF RATS AND ON BRAIN CATECHOLAMINE METABOLISM OF RATS DIFFERING IN THEIR LEVEL OF EMOTIONAL-BEHAVIORAL REACTIVITY

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UDC 616.89-008.46-02:615.31:547.583.5]-092.9-07

KEY WORDS: Madopar; L-dopa; emotional-behavioral reactivity; catecholamine metabolism.

Differences in the neurochemical characteristics of inbred animals determine individual variability of the effects of drugs [10]. Among the population of noninbred animals (rats) groups differing significantly in sensitivity to psychotropic drugs also are distinguished, and this is associated with individual variations in catecholamine (CA) metabolism [2]. This is essential when the neurochemical mechanisms responsible for behavioral disturbance following injection of L-dihydroxyphenylalanine (L-dopa) — a dopamine (DA) precursor are studied. The aim of this investigation was to study L-dopa-induced changes in the behavior of rats differing in their level of emotional-behavioral reactivity (EBR) and concentrations of L-dopa, CA, and dihydroxyphenylacetic acid (DOPAA) in their brain structures.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-220 g. The animals were selected according to their EBR level on the basis of qualitative and quantitative parameters of behavior in the open field test (OF), in freely revolving drums (FRD), and in the extrapolation avoidance test (EAT) [1]. In OF the number of rearings in the central zone, the number of crosses from one square to another, the number of holes sniffed, and the number of acts of grooming were estimated; in FRD locomotor activity was assessed (in meters during 10-min intervals for 30 min after placement), and in EAT the latent period (LP) of motor activity, the number of attempts to run away, and LP of avoidance. Animals of two batches of identical composition (each consisting of groups of rats A and B differing in their EBR level) were given an interperitoneal injection, 3 days after testing, of 0.9% NaCl (control batch) or of the preparation Madopar-125 (from Galenika, Yugoslavia) in a dose of 150 mg/kg (which contains 100 mg of L-dopa and 25 mg of benserazide, an inhibitor of peripheral aromatic amino-acid decarboxylase), and 1 h later, half of the animals from the A and B groups (eight rats in each case) of each batch were again tested and the remaining half of the animals killed by decapitation.

Concentration of L-dopa, DA, noradrenalin (NA), and DOPAA in the brain structures of the rats were determined by high-pressure liquid chromatography with electrochemical detection [6]. The results were subjected to statistical analysis by the Wilcoxon—Mann—Whitney test.

EXPERIMENTAL RESULTS

Animals of group B differ from rats of group A in a number of behavior parameters (Table 1). The intensity of biased activity (the number of acts of grooming), reflecting conflict

Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 8, pp. 168-170, August, 1988. Original article submitted April 15, 1987.

