DISTRIBUTION OF ^{14}C-HYDROGENATED ANALOG OF PHENAZEPAM IN SOME MOUSE ORGANS AND TISSUES

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The most frequent side effects of tranquilizers of the benzodiazepine series are disturbance of movement coordination and muscular weakness. This has led to the study of the possibility of obtaining preparations with reduced side effects on muscle tone, or what are known as 24-hour tranquilizers. There is evidence [8] that some derivatives of 1,4-benzodiazepines, although inferior to other preparations in their tranquilizing properties, nevertheless give rise to a much weaker hypnotic and muscle-relaxing effect [1, 6]. The present writers have shown [4] that one such substance is the hydrogenated analog of phenazepam (I).

This paper gives data on the distribution of ^{14}C-I (labeled in position 2 of the heterocyclic ring) in some organs and tissues of mice receiving a single injection of the compound.

EXPERIMENTAL METHOD

^{14}C-I was synthesized by the method of Rudenko et al. [6, 7]. At all stages or preparation of ^{14}C-I the chemical purity of the intermediate products was verified by thin-layer radiochromatography. Quantitative analysis demonstrated a high degree of incorporation of the reagents during synthesis. Total and specific radioactivity of the substances in the test samples were recorded on an L5100 liquid scintillation photometer (from Beckman, USA).

Experiments were carried out on male F_1 mice weighing 18-24 g, into which ^{14}C-I was injected intraperitoneally in a single dose of 10 mg/kg body weight in the form of an emulsion in Tween-40. The animals were decapitated 16, 30, and 60 min and 3, 6, 12, and 24 h later. Samples of liver, brain, blood plasma, kidney, spleen, lung, heart, and muscle and adipose tissues were homogenized and subsequently treated as in [3].

By means of a series of model experiments, the methods of extraction and determination of ^{14}C-I in the biological samples were optimized. The most suitable condition for quantitative extraction of ^{14}C-I is pH between 7.0 and 8.0. Under these circumstances no dependence was observed of the degree and kinetic parameters of extraction on the ^{14}C-I concentration in the sample or the nature of the biological substrate. However, addition of ^{14}C-I to a solution of albumin reduced the possibility of extraction of the test substance [7]. The numerical results were subjected to statistical analysis [5].

EXPERIMENTAL RESULTS

Analysis of the concentration of radioactive material revealed comparatively rapid entry of ^{14}C-I into the organs and tissues of the experimental animals. When 15 min had elapsed after injection of the substance, much of it was found in the liver, blood plasma, kidneys, spleen, and adipose tissue (Table 1). However, maximal values of this parameter were found between 30 and 60 min after injection, with only a very small increase toward

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TABLE 1. Distribution of Total Radioactive Material in Organs and Tissues of Mice After Injection of ^{14}C-I

1 Test object	Time						
	15 min	30 min	1 h	3 h	6 h	12 h	24 h
Liver	25,5±1,0	30,9±1,9	33,0±1,0	24,1±1,3	16,3±1,1	10,6±3,7	5,5±0,5
Brain	4,4±0,4	9,6±0,3	11,5±0,4	9,1±0,5	5,2±0,6	3,4±0,4	1,9±0,3
Plasma	8,9±0,6	9,8±0,9	20,1±0,2	16,0±0,1	11,7±1,9	8,8±0,7	4,9±0,4
Kidneys	11,9±0,4	16,2±0,7	16,5±0,7	12,4±1,2	8,3±0,4	3,7±0,2	2,1±0,2
Spleen	15,6±0,9	13,9±0,7	14,8±1,6	9,9±0,5	5,3±0,5	3,2±0,2	1,3±0,1
Lungs	7,8±0,2	10,6±0,9	10,8±0,6	8,9±0,4	5,4±0,4	1,8±0,2	0,8±0,1
Heart	6,1±0,1	12,7±0,3	13,3±0,6	9,8±0,6	6,1±0,5	3,2±0,2	1,7±0,1
Muscle tissue	5,0±0,4	7,1±0,5	6,9±0,8	4,7±0,3	3,4±0,2	1,9±0,3	0,6±0,1
Adipose tissue	31,1±3,1	—	34,5±3,2	23,2±3,5	12,3±0,7	8,1±0,9	4,3±0,6

Legend. Content of radioactive label in organs and tissues expressed in cpm × 10^{-3}/g tissues, in plasma in cpm × 10^{-3}/ml.

the 60th minute. The high level of radioactive material in the liver, blood plasma, and kidneys was evidently associated with their metabolic functions, whereas the presence of ^{14}C-I in adipose tissue can be explained by the high lipophilicity of the original compound [5], which is also characteristic to some degree of phenazepam [1]. A significant difference in the rats of elimination of ^{14}C-I from adipose tissue and blood plasma was observed only between 1 and 6 h after injection; later the elimination process followed a parallel course, indicating a high level of exchange between these tissues. Consequently, adipose tissue cannot be regarded as a region of slow metabolism. Equally, the absence of deposition of the drug may apply also the other tissues studied, for no biosubstrate could be found in which the fall in the level of radioactive material took place less rapidly than in blood plasma, and with lengthening of the time of the experiment the ratio between the rates of elimination in the blood plasma–tissue system gradually approached a constant value (Table 1).

The smallest quantity of radioactive label was found in the muscles, most in the liver, and the remaining tissues occupied an intermediate position; 24 h after injection of the substance, the label was present in all tissues.

Comparison of the distribution of ^{14}C-phenazepam with the corresponding parameters for ^{14}C-I showed differences in the time when maximal concentrations of these substances occurred in the mouse tissue. The highest level of radioactivity for phenazepam was observed after 30 min [2]. After injection of ^{14}C-I a slower process of accumulation was observed in all the biological substrates examined, with a peak after 60 min. Another fact of great importance is the ratio between the concentrations of psychotropic drugs in the animal's blood plasma and brain, which may be connected with tis pharmacologic activity. After injection of phenazepam, its level was higher in the brain than in the blood plasma, whereas after injection of ^{14}C-I the plasma level was higher. This rule was maintained throughout the duration of the experiment.

It can be concluded from analysis of the kinetic parameters of distribution or, more precisely, the fall in the level of radioactive material in the mice that elimination of the original compound and of its metabolites is a biexponential process, consisting of fast and slow phases. The fast phase of elimination of ^{14}C-I, moreover, is recorded between 1 and 6 h after injection for most tissues and organs, and the level of radioactive label falls to 50-60% of its initial value. The only exceptions are tissues of the kidneys, heart, and lungs, in which the duration of fast exchange is 1-12 h. For the fast phase the half-elimination time ($T_{1/2}$) of the labeled compound is 4-5 h, and $T_{1/2}$ for the slow phase is 9-12 h. For elimination of ^{14}C-I from blood plasma $T_{1/2}$ = 6.5 h in the first case and $T_{1/2}$ = 15.5 h in the second case. In both phases dependence of the rate of elimination of ^{14}C-I on time is linear.

On the basis of the data and of comparison of the results of a study of the stages of accumulation and elimination of ^{14}C-phenazepam and ^{14}C-I it can be concluded that there are differences in the intensity of metabolism of these compounds in experimental animals. The rate of entry of ^{14}C-I into the tissues and organs of mice is slower than the rate of entry of ^{14}C-phenazepam. Elimination of ^{14}C-phenazepam and ^{14}C-I is biphasic in character, but the second compound is eliminated more slowly.

LITERATURE CITED

1. N. Ya. Golovenko and V. G. Zin'kovskii, in: Phenazepam [in Russian], Kiev (1982), pp. 32-86.
2. N. Ya. Golovenko, V. G. Zin'kovskii, and S. B. Seredenin, Khim.-Farm. Zh., No. 12, 14 (1980).
3. N. Ya. Golovenko, V. G. Zin'kovskii, S. B. Seredenin, et al., Farmakol. Toksikol., No. 2, 144 (1980).
4. N. Ya. Golovenko, O. P. Rudenko, T. L. Karaseva, et al., "Pharmacologic properties of tetrahydrophenazepam 7-bromo-5-phenyl-(0-chloro)-1,3,4,5-tetrahydro-2H-1,4-benzodiazepan-2-one," paper deposited at VINITI, April 6, 1983, No. 1805-83 Dep.
5. E. Piotrowski, The Use of Kinetics of Metabolism and Elimination of Toxic Substances in the solution of Problems in Industrial Toxicology [in Russian], Moscow (1976).
6. O. P. Rudenko, T. L. Karaseva, A. I. Lisitsyna, et al., Author's Certificate No. 1051081 (USSR).
7. V. A. Sozinov, O. P. Rudenko, V. G. Zin'kovskii, et al., Khim.-Farm. Zh., No. 10, 1232 (1984).
8. L. Otvös, Z. Tegyey, L. Vereczkey, et al., Drug Metab. Dispos., 6, 213 (1978).

MECHANISM OF THE TRANQUILIZING ACTION OF SOME ELECTRONIC STRUCTURAL ANALOGS OF NICOTINAMIDE

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Nicotinamide, hypoxanthines, β-carbolines, and various other endogenous brain compounds are probable endogenous ligands of benzodiazepine receptors (BDR) [11, 12, 14, 15]. It has been shown that nicotinamide possesses certain pharmacologic properties on the basis of which it can be regarded as a tranquilizer [1, 2, 4, 8, 9]. It is interesting in this connection to search for compounds with tranqilizing activity, superior to that of nicotinamide, among its analogs (amides of aminonicotinic, hydroxynicotinic, and hydroxyisonicotinic acids, esters of nicotinic acid) with definite electronic structural similarity with tranquilizers of the benzodiazepine series [7].

With this aim, and using a model of a conflict situation and the technique of radioligand analysis, the writers investigated two original compounds (coded NMF and AzN), which are ethyl esters of nicotinic acid. Nicotinamide itself and inosine, another hypothetical ligand of BDR, were used as substances for comparison.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-230 g. The anxiolytic effect of nicotinamide (250-500 mg/kg), inosine (500 mg/kg), NMF, and AzN (20 mg/kg) was studied under conditions of a conflict situation [3, 5]; the substances were injected intraperitoneally 30-40 min before testing. Participation of GABA-ergic mechanisms in the realization of the anxiolytic action of the compounds was demonstrated by the use of specific analyzers: calcium valproate (200 mg/kg) and bicuculline (1 mg/kg), which were injected 40 and 5 min respectively before the experiment. To discover the role of central BDR, the specific antagonist of benzodiazepine receptors Ro 15-1788 was used in a dose of 10 mg/kg, given 10 min before testing. The effect of the test substances, in concentrations

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