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BIOKINETICS OF A NEW PROTODRUG HYDAZEPAM AND ITS METABOLITE

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UDC 615.213:547.631.6.015.4.07

KEY WORDS: hydazepam; protodrug; physiologicallyactive metabolite; effector analysis of pharmacokinetics

Hydazepam (I) [1-(hydrazinocarbonyl)-7-bromo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one] is a new original therapeutic preparation of benzodiazepine structure, recommended for clinical use as a tranquilizer and combining an anxiolytic and anticonvulsant action with mildness of its side effects and with low toxicity. In experimental animals I undergoes intensive N^1-dealkylation with the formation of a physiologically active metabolite, namely 7-bromo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one (II) and products of its further oxidation [5]. The affinity of II for benzodiazepine receptors in the CNS is higher (by more than 3 orders of magnitude) than that of I. Accordingly, I can be regarded as a protodrug, and II as a drug-metabolite [4].

The aim of this investigation was to substantiate methods of effector analysis of the pharmacokinetics of I, and to determine the biokinetic features of the protodrug (I) and to compare them with the corresponding parameters of its physiologically active metabolite (II).

EXPERIMENTAL METHOD

Experiments were carried out on female CBA mice weighing 18-22 g – intact mice and mice receiving various doses (0.7-22.4 mg/kg) of 2-^{14}C-I (0.27 Ci/mole) intraperitoneally within a time interval (0.083-24 h) of the experiment. Minimal effective doses of metrazol (mg/kg) inducing the development of clonicotonic convulsions (DCTC) and tonic extension (DTE) [8, 11], by intravenous infusion (0.01 ml/sec) of a 1% solution into the caudal vein, were determined as the recorded parameters of the pharmacodynamics. Parallel determinations were made of concentrations of ^{14}C-products in the animals' brain. The methods of determination of ^{14}C-compounds in the mouse brain during simultaneous recording (±1 min) of the values of DCTC and DTE, were described previously [3, 6]. Concentrations of ^{14}C-products in the blood plasma and brain of the mice were determined within the interval of 0.017-24 h and 0.017-6 h of the experiment after intraperitoneal injection of ^{14}C-I and ^{14}C-II (0.70 and 0.78 Ci/mole) intraperitoneally into the animals in doses of 1.4 mg/kg, by methods described in [5, 7]. The experimental data were analyzed using algorithms described in [2, 10].

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Research Problem Laboratory No. 5, I. M. Mechnikov Odessa State University. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 1, pp. 45-47, January, 1992. Original article submitted July 26, 1990.

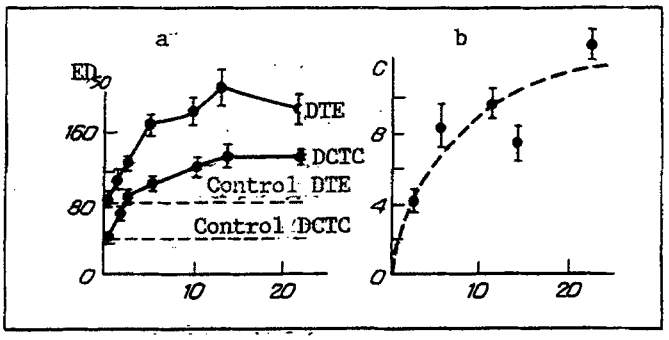

Fig. 1. Dynamics of anticonvulsant effect (a) – change in values of minimal effective doses (mg/kg) of metrazol inducing clonicotonic convulsions (DCTC) and tonic extension (DTE) mice – change in concentration of ^{14}C-products (counts $\cdot 10^3$/min $\cdot$ g) in animals' brain (b) 2 h after injection of increasing doses (mg/kg) of ^{14}C-hydazepam (0.27 Ci/mole).

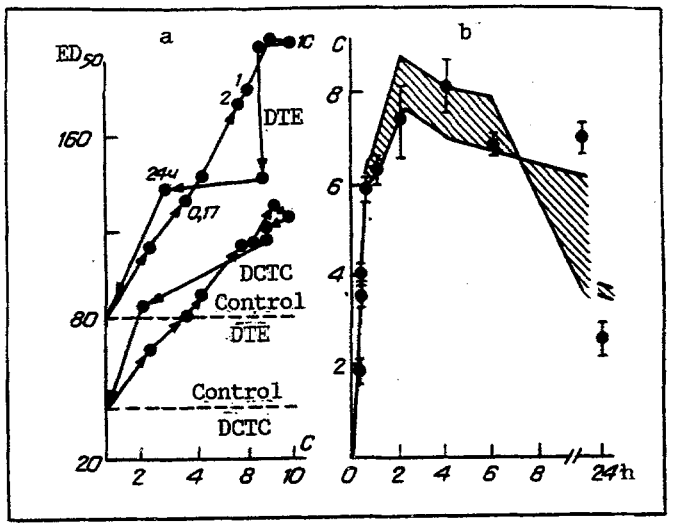

Fig. 2. Dependence of dynamics of anticonvulsant effect (ordinate) on content of ^{14}C-products in brain of mice (abscissa) within 0.17-24 h time interval after injection of ^{14}C–I in a dose of 1 mg/kg (a). Legend as to Fig. 1. Comparison of results of pharmacokinetic analysis (M ± m) and results of effector prognosis of concentration of ^{14}C-products in biophase of action of I (b) (interval of disagreement between results of effector prognoses shaded; conditions analogous to Fig. 2a).

EXPERIMENTAL RESULTS

Forms of the dose–effect curves plotted in experiments for the protodrug (I) (equation 1) and its metabolite (II) [9] were similar: injection of increasing doses (di) of ^{14}C-I led (Fig. 1a) to a limited (hyperbolic) increase in the values of DCTC and DTE compared with their control values:

$$ED_{50,i} - ED_{50,к} = (ED_{50,м} - ED_{50,к}) \cdot (1 + d_{50} \cdot d_i^{-1})^{-1}, \quad (1)$$

where $ED_{50,m}$, $ED_{50,i}$, and $ED_{50,max}$ denote values of minimal effective doses of metrazol (DCTC and DTE) in the groups of intact animals after receiving an injection of a dose d_i of I (calculated values for $d_i \to \infty$); d_{50} denotes half the effective doses of I inducing an anticonvulsant effect corresponding to 0.5 of maximal: $(ED_{50,max} - ED_{50,m}) \times 0.5$.

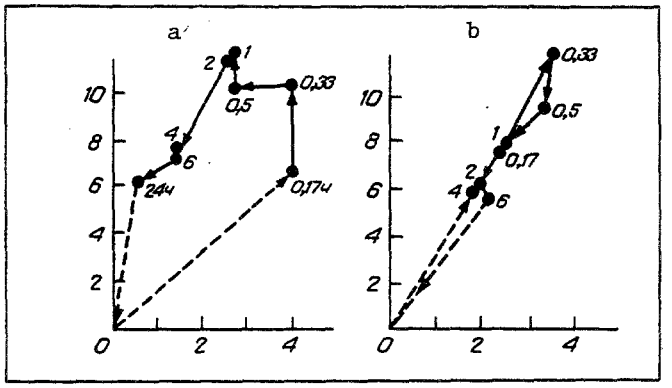

Fig. 3. Changes in concentration (counts $\cdot 10^3$/min $\cdot$g) of ^{14}C-products in blood plasma (abscissa) and brain (ordinate) of mice after receiving injection of protodrug (2-^{14}C—I, 0.78 Ci/mole) (a) and its metabolite (2-^{14}C-II, 0.78 Ci/mole) (b). Numbers indicate time interval (h) after injection.

Values of d_{50} of hydazepam based on values of DCTC and DTE were found to be 2.98 ± 0.33 and 2.3 ± 0.45 mg/kg, significantly ($p \leq 0.01$) lower than the corresponding values for the pharmacodynamics of its physiologically active metabolite II [6]. A significant feature of the pharmacodynamics of I was the nonlinear (hyperbolic, unlike with II) increase in the concentration of ^{14}C-products in the mouse brain after injection of increasing doses of the protodrug

$$C_i = C_{i\,max}\, d_i (d'_{50} + d_i)^{-1}, \qquad (2)$$

where C_{max} denotes the maximal concentration (counts × 10^3/min $\cdot$g) of ^{14}C products in the biosubstrate after injection of dose d_i of ^{14}C-I ($d_i \to \infty$); d'_{50} denotes the dose of ^{14}C—i for which $C_i = C_{max} \times 0.5$, determined as 7.1 ± 0.29 mg/kg.

On the basis of determination of the form of correlation between the dynamics of the anticonvulsant effect and the kinetics of the concentration of ^{14}C-products in the mouse brain (Fig. 2a) it can be concluded that the "effector" compartment and the biophase of the action of I may be combined into a single kinetic chamber (compartment), in view of the equivalence of the "concentration—effect" relationship. This relationship is expressed by a linear function (Fig. 2a) (with satisfaction of the condition: $d_{50} \approx d'_{50}$), by contrast with this relationship for the metabolite (II) [9]. The "dose—effect" and "dose—concentration" functions discovered allow effector analysis of the pharmacokinetics of I to be undertaken 2b). As the results of analysis show (Fig. 2b) the effector prognoses of the concentration of ^{14}C-compounds in t brain, based on values of DCTC and DTE, are in agreement with one another and with the level of concent ^{14}C-products within the 0.083-0.5 h time interval of the experiment, whereas within the 1-6 h interval disagree between effector prognoses (shaded sectors) are acceptable (<0.15) and are comparable with the results of pharmacokinetic (radiometric) analysis. The significant differences in the kinetics of the concentration of ^{14}C-products in the brain after injection of hydazepam and its metabolite into the mice must be noted. In the first case the rapid phase of distribution of I was not observed, the maximal level of the concentration of II was reached sooner (after 0.25-0.33 h), and elimination from the biological substrate tested took place more rapidly [7, 9]. The differences in the biokinetics of I and II are as follows: after injection of I into the animals the ratio of the concentration of ^{14}C-products in the blood plasma and brain changed regularly, demonstrating hysteresis during the time of the experiment, and this suggests that the blood plasma corresponds to the central, and the brain to the peripheral compartments of the kinetic scheme of distribution of I in the body (Fig. 3a). After injection of the metabolite into the animals (Fig. 3b) a linear relationship was observed, unchanging with the time of the experiment, suggesting that the two biological substrates function as a single (central) compartment of the kinetic scheme of distribution of II. This conclusion (Fig. 3a, b) is important for the planning and conduct of clinical trials, in which blood plasma is used as the test object for the pharmacokinetics of the protodrug (I) and its metabolite (II).

One result of the nonlinearity of the "dose—concentration in the biophase of action" relationship of the kinetic scheme of distribution of I (Fig. 1a) is the relatively low concentration of ^{14}C-products (reaching a maximum 4 h after injection) in the animals' brain after injection of high doses of the protodrugs (14 mg/kg; Fig. 2b) and the more rapid (0.5-2 h) achievement of the peak level of labeled products in the biophase of action (Fig. 3a) after injection of II in low doses (1.4 mg/kg).

TABLE 1. Characteristics of Basic Processes of Biokinetics of Protodrug (I) and Its Physiologically Active Metabolite (II)

Dependence		
Type	Form	
	I	II
"Dose – effect"	Hyperbolic	Hyperbolic
"Dose – concentration"	Hyperbolic	Linear
"Concentration – effect"	Linear	Hyperbolic
Ratio of concentration "blood plasma/brain"	Variable	Constant
Structure of scheme of biokinetics	I	II
Effector compartment and "biophase of action" (brain)	Single compartment (peripheral)	Single compartment (central)
"Biophase of action" (brain) and blood plasma	Peripheral and central compartments	Single compartment (central)
Effector compartment and blood plasma	Peripheral and central compartments	Single compartment (central)

Comparison of the basic characteristics of the biokinetics of the two compounds studied (Table 1) explains the nature of the differences in their pharmacologic action and, consequently, indicates the possibility of their use as therapeutic preparations.

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