in BP against the background of naloxone may perhaps reflect one of its actions and may explain its antishock properties, for elevation of BP has been demonstrated under the influence of naloxone in spinal, endotoxin, and other types of shock [8, 13, 14]. It can be tentatively suggested that electrolytic decerebration may activate the release of endogenous opiate peptides, whose effect is blocked by the naloxone administered later. Considering our own data and the results of experiments conducted by Farsang et al. [9, 10], it is suggested that an endogenous opiate mechanism is of great importance for the regulation of the background parameters of the hemodynamics, but it evidently does not participate in processes of somato-autonomic stress.

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GENETIC DIFFERENCES IN THE ANTISEIZURE EFFECT AND METABOLIC RATE OF PHENAZEPAM

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Previous experiments have shown that the antiseizure effect of phenazepam and the rate of its oxidation *in vitro* are genetically dependent [1, 2]. The object of this investigation was to study the metabolism of the tranquilizer *in vivo* in C57BL/6 and BALB/c mice and also the character of inheritance of the antiseizure effect and the metabolic rate of the drug in first generation hybrids.

EXPERIMENTAL METHOD

Male inbred mice of lines C57BL/6 (B6) and BALB/c (C) lines and (C × B6)F_1 hybrids weighing 18-20 g were used in the experiments. To study metabolism, ^{14}C-phenazepam was injected intraperitoneally with Tween-80 in a dose of 14 mg/kg. The animals were decapitated 0.5, 1, 2, 3, and 6 h after injection of the substance. The total radioactivity of the initial compound (I), of its 3-hydroxy metabolite (II), of the total derivatives hydroxylated in the aromatic rings of the phenazepam molecule (III), and protein-bound radioactivity (IV) in the blood plasma, brain, and liver were determined [3]. Antiseizure activity of compound I against metrazol was estimated by the method described previously [2]. For statistical analy-

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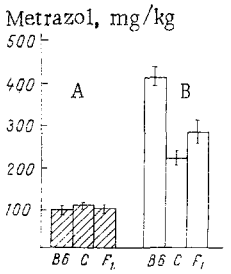

Fig. 1. Antiseizure activity of phenazepam in C57BL/6 (B6), BALB/c (C), and (C × B6)F_1 (F_1) mice to metrazol 30 min after intraperitoneal injection of phenazepam in a dose of 1.75 mg/kg. A) Control, B) phenazepam.

sis of the data, Student's (t_α) and Fisher's (F) criteria were used. If the latter exceeded values in the tables at the $P < 0.05$ level the number of degrees of freedom (f) was calculated by the equation:

$$f = \frac{(n_1 - 1)(n_2 - 1)}{(1 - u^2)(n_1 - 1) + u^2(n_2 - 1)}, \quad u = \frac{m_1^2}{m_1^2 + m_2^2}.$$

To assess interlinear differences in the metabolic rate of the tranquilizer, methods of dispersion analysis were used. The criterion of interfactorial dispersion t_α proved to be effective for comparison and comparable with the value of t_α. The index of strength of the effect of a hereditary factory η_A was determined for its unbiased values.

EXPERIMENTAL RESULTS

Radiochromatographic analysis revealed the presence of compounds I, II, III, and IV in the biological substrates from B6 and C animals. No qualitative differences were found in the conversions of the tranquilizer. Meanwhile differences were found in the metabolic rate of compound I, most marked at the hydroxylation stage in position 3. These results are given in Table 1.

The experiments *in vivo* thus confirmed data obtained *in vitro* indicating a lower rate of oxidation of compound I in B6 mice [1], coupled with the greater sensitivity of this genotype to the protective action of the tranquilizer when titrated against metrazol [2]. The antiseizure effect of compound I on (C × B6)F_1 hybrids was equally strong as on mice of the parental C line, but significantly weaker than on B6 mice (Fig. 1). The study of metabolism of compound I in the hybrid animals showed the presence of derivatives found in both B6 and C. The most significant quantitative differences were observed in the rate of hydroxylation of the compound I molecule in position 3, and for that reason the levels of compounds I and II were chosen in order to compare metabolism of the tranquilizer in B6 and C mice and their F_1 hybrids. It will be clear from Tables 2 and 3 that the differences in the concentration of these substances in the biological substrates of (C × B6)F_1 and B6 mice were similar in most cases to differences found in B6 and C mice (Table 1).

The index of the strength of the effect of hereditary factors η_A^2 on the content of compound I after 0.5-1 h in the brain of B6 and C mice was 27-37%, in (C × B6)F_1 and B6 mice 36-49%, and in (C × B6)F_1, B6, and C mice 37-38%. No significant differences were found between the hybrid and C mice. Dependence of the level of compound II in the brain on genotype was even more marked 1 and 2 h after administration of the drug: η_A^2 for B6 and C varied between 48 and 77%, for (C × B6)F_1 and B6 53-64%, for (C × B6)F_1, B6, and C 43-68%. No differences at these times likewise were found between (C × B6)F_1 and C mice.

In the liver, which is responsible for converting compound I into compound II, their concentrations were similar in C and (C × B6)F_1 mice and differed significantly from their values in B6 mice throughout the experiment.

TABLE 1. Distribution of Compounds I and II (in cpm × 10^{-3}) in B6 and C Mice (n = 5) after Administration of Tranquilizer in Dose of 14 mg/kg (M ± m)

Organ	Time, h	Compound I		t_d	Compound II		t_d
		B6 mice	C mice		B6 mice	C mice	
Liver	1/2	147,7±38,1	264,7±37,5	2,19	12,7±3,6	51,4±6,4	5,22†
	1	241,9±14,7	229,8±33,5	0,33	21,3±8,6	54,3±9,2	2,62*
	2	185,6±12,1	162,8±14,6	1,20	38,5±4,6	58,1±6,4	2,47*
	3	153,8±22,7	133,5±13,2	0,77	51,0±6,7	57,9±4,0	0,88
	6	122,5±9,2	40,4±11,2	5,67†	61,6±9,3	65,2±7,2	0,31
Brain	1/2	64,9±13,2	101,9±10,9	3,19*	3,6±0,9	7,1±1,3	2,28
	1	122,5±13,9	82,9±7,6	2,31*	6,3±0,9	12,1±0,6	5,48†
	2	75,8±4,6	75,5±7,0	0,04	8,8±1,0	17,3±2,4	3,22*
	3	70,4±7,8	51,4±6,7	1,86	14,4±2,2	17,4±2,4	0,90
	6	56,7±12,1	19,2±3,9	2,96*	18,7±3,0	19,1±4,2	0,08
Plasma	1/2	36, ±4,8	131,1±14,1	6,33†	3,1±0,7	22,4±4,3	4,44†
	1	82,1±4,1	50,9±5,1	4,79†	3,2±0,3	13,0±1,0	9,12†
	2	—	43.6±1,7	—	7,5±1,1	14,9±0,6	5,84†
	3	52,3±8,9	37,3±4,1	1,53	14,6±2,6	16,3±2,0	0,48
	6	33,0±1,8	9,2±1,1	11,05†	21,2±1,7	12,2±1,7	3,77†

Legend. t_d) Student's coefficient of significance of differences between By and C animals; here and in Tables 2 and 3.
*P < 0.05.
†P < 0.01.

TABLE 2. Content of Compound I (in cpm/g × 10^{-3}) in (C × B6)F_1 Hybrids (n = 5) after Administration of Tranquilizer in Dose of 14 mg/kg (comparison with parental lines)

Organ	Time, h	Compound I			A		B		C		D	
		F_1 (M±m)	^1t_d	^2t_d	F_φ	η^2_A, %	F_φ	η^2_A, %	F_φ	η^2_A, %	F_φ	η^2_A, %
Liver	1/2	217,5±14,2	1,17	1,72	—	—	—	—	—	—	—	—
	1	188,0±19,1	1,09	2,39*	—	—	—	—	—	—	—	—
	2	146,0±6,9	1,04	2,84*	—	—	8,08	44,0	—	—	—	—
	3	111,2±8,3	1,42	1,93		—	—	—	—	—	—	—
	6	57,2±3,7	1,51	6,58†	—	—	43,4	82,5	25,2	77,6	32,08	77,5
Brain	1/2	111,3±5,3	0,78	3,25*	—	—	9,73	49,2	5,82	38,2	4,39	27,3
	1	83.7±7,1	0,06	2,48*	—	—	6,11	36,2	5,02	36,6	6,22	36,7
	2	61,2±5,1	1,63	2,14	—	—	—	—	—	—	—	—
	3	56,6±5,2	0,59	1,48	—	—	—	—	—	—	—	—
	6	32,5±2,1	3,07*	1,57	9,02	47,1	—	—	6,53	44,1	8,70	46,1
Plasma	1/2	73,1±3,6	3,98†	6,04†	15,89	62,3	37,01	80,3	29,02	80,0	40,25	81,3
	1	47,4±4,9	0,52	5,43†	—	—	29,50	76,0	16,38	68,7	22,73	70.7
	2	35,6±2,9	1,15	—	—	—	—	—	—	—	—	—
	3	33,3±1,6	0,18	2,09	—	—	—	—	—	—	—	—
	6	21,4±2,0	5,26†	4,31†	28,57	75,6	18,59	61,1	50,30	87,6	127,3	93,3

Legend. Here and in Table 3, ^1t_d) Student's coefficient of significance of differences between C and F_1 animals; ^2t_d) Student's coefficient of significance of differences between B6 and F_1 animals; A) indices of dispersion analysis of differences between C and F_1 animals, B) indices of dispersion analysis of differences between B6 and F_1 animals, C) indices of dispersion analysis of differences between B6, C, and F_1 animals, D) indices of dispersion analysis of differences between B6 and C animals.

TABLE 3. Content of Compound II (in cpm/g × 10^{-3}) in (C × B6)F_1 Hybrids (n = 5) after Administration of Tranquilizer in Dose of 14 mg/kg (comparison with parental lines)

Organ	Time, h	Compound II			A		B		C		D	
		F_1 ($M \pm m$)	^1t_d	^2t_d	F_φ	η^2_A, %	F_φ	η^2_A, %	F_φ	η^2_A, %	F_φ	η^2_A, %
Liver	1/2	29,7±2,8	3,08*	3,71†	9,65	49,0	13,89	58,9	18,28	71,2	15,28	61,3
	1	45,8±4,6	0,81	2,53*	—	—	6,31	37,1	4,90	35,8	6,87	39,5
	2	57,3±3,1	0,11	3,48†	—	—	11,49	53,8	9,90	48,8	13,89	58,9
	3	59,1±6,1	0,17	1,52	—	—	—	—	—	—	—	—
	6	45,8±4,6	1,13	1,95	—	—	—	—	—	—	—	—
Brain	1/2	6,1±1,0	0,57	1,95	—	—	—	—	—	—	—	—
	1	10,6±0,5	0,66	4,22†	—	—	17,44	64,6	20,76	68,4	31,74	77,4
	2	14,8±1,5	0,88	3,32†	—	—	11,08	52,8	6,35	43,3	10,67	48,2
	3	20,9±5,1	0,62	0,16	—	—	—	—	—	—	—	—
	6	19,2±3,9	0,03	0,11	—	—	—	—	—	—	—	—
Plasma	1/2	9,5±0,8	2,95*	5,75†	8,70	46,1	36,25	79,7	14,78	32,7	19,63	67,4
	1	9,0±0,8	4,91†	6,99†	10,4	53,0	37,70	82,1	37,59	85,9	88,11	92,6
	2	12,0±1,8	1,49	2,09	—	—	—	—	8,67	52,3	34,88	79,0
	3	15,4±1,7	0,35	0,23	—	—	—	—	—	—	—	—
	6	14,7±1,4	1,11	2,97*	—	—	8,45	45,3	8,17	45,3	13,70	58,5

The concentration of compounds I and II in the blood plasma of the hybrid animals after 0.5, 1, and 6 h was characterized by intermediate values between those of the parental lines; however, η^2_A for (C × B6)F_1 and C mice was lower than for (C × B6)F_1 and B6 mice.

Conversion of compounds I and II in F_1 hybrids thus bears a closer resemblance to the same process in C animals.

Comparison of the results of investigation of the antiseizure action of compound I and its metabolism reveals negative correlation between genetically determined sensitivity to the drug and the rate of its oxidation. Low sensitivity, like high metabolic activity, is evidently inherited as a dominant trait, or incomplete dominance is manifested. It is too early to draw and final conclusions on the causes responsible for these facts, for there is evidence of differences in the number of benzodiazepine receptors in the strains of mice used in the investigation [4].

Meanwhile the possibility cannot be ruled out that the two hereditary factors play a role in the formation of the antiseizure effect of the tranquilizer. In view of the possibility that similar genetic principles may be involved in the human population also, there is good reason to recommend pharmacokinetic surveillance of the use of compound I, especially in the treatment of seizure states.

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