In the experiments of series IV the effect of naloxone in a dose of 0.1 mg/kg on BP was studied in four reserpinized rabbits. The initial BP in the femoral artery was 72.4 ± 5.6/67.7 ± 5.2 mm Hg. BP fell 2-3 h after injection of reserpine in a dose of 2.5 mg/kg to 35.4 ± 4.4/30.2 ± 3.9 mm Hg. During this period the animals were given naloxone in a dose of 1.1 mg/kg. No changes in BP were observed in response to injection of naloxone.

It has been shown that under the influence of stressors, including nociceptive stimuli, an increase in the concentration of endogenous opioid peptides, especially β-endorphin, is observed in the blood of animals (rats [3], rabbits [5]). These substances are known to be capable of inhibiting various autonomic systems of the body, including the cardiovascular and respiratory systems [2]. These facts suggest that endogenous opioid peptides may play an important role in the formation of disturbances of activity of autonomic systems under the influence of shock-producing factors. This is shown by blocking of the development of electronociceptive shock observed in rabbits in the present experiments, and also by results indicating an improvement in the state of rats during hypovolemic and endotoxic shock, and in dogs with hypovolemic shock after administration of antagonists of endogenous opioid peptides [1, 4]. Injection of naloxone into animals with marked hypotension, due not to the shock-producing factor but to injection of reserpine, was ineffective.

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THE USE OF DISPERSION ANALYSIS TO ESTIMATE THE ANTICONVULSANT ACTIVITY OF 1,4-BENZODIAZEPINES IN MICE

Academician A. V. Bogatskii,* O. V. Zhuk, V. G. Zin'kovskii, and N. Ya. Golovenko

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KEY WORDS: derivatives of 1,4-benzodiazepines; metrazol convulsions; dispersion analysis.

The object of this investigation was to study the time course of changes in the convulsant action of metrazol following injection of 1,4-benzodiazepine derivatives, with different chemical structure, into mice. To assess the significance of the effect of the time factor and the structure of the compounds used on the effect formed by them, the experimental results were subjected to dispersion analysis [2, 4].

EXPERIMENTAL METHOD

Experiments were carried out on 213 male CBA mice weighing 18-24 g. The animals were given 1,4-benzodiazepines (phenazepam and its 3-hydroxy derivative, demethyldiazepam, demethylsulazepam) in a dose of 5 mg/kg in Tween emulsion. Control animals (25 mice) received an aqueous solution of Tween-80. Minimal doses of metrazol which, when injected into the caudal vein of mice 5, 15, 30, and 120 min after injection of the test compounds, caused pseudoclonic twitches (DPCT), clonicotonic convulsions (DCTC), and tonic extension (DTE) were established; a 1% solution of metrazol was injected at the rate of 0.01 ml/sec [1].

*Academician of the Academy of Sciences of the Ukrainian SSR.

Laboratory of Psychotropic Drugs, I. I. Mechnikov Odessa University.

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TABLE 1. Changes in Values of DPCT, DCTC, and DTE depending on Time of Experiment after Intraperitoneal Injection of 1,4-Benzodiazepine Derivatives into Male Mice in a Dose of 5 mg/kg (M ± m)

Time, min	Compound	DPCT	DCTC	DTE
5	1	118,7±4,75 (12)	181,6±6,11 (12)	300,8±13,9 (12)
	2	87,0±4,69 (12)	146,9±7,7 (12)	234,5±11,1 (12)
	3	110,3±3,3 (9)	141,7±6,6 (9)	192,0±12,2 (9)
	4	88,3±3,3 (9)	122,3±4,7 (9)	150,5±6,3 (9)
15	1	136,9±9,72 (17)	232,0±12,1 (17)	375,8±14,1 (16)
	2	91,3±3,1 (11)	147,7±12,3 (11)	240,6±15,0 (11)
	3	125,1±5,5 (9)	179,4±10,3 (9)	245,1±12,4 (9)
	4	98,5±4,2 (10)	129,6±7,3 (10)	173,9±8,3 (10)
30	1	115,9±4,1 (10)	195,9±6,4 (14)	339,0±14,2 (13)
	2	87,9±4,1 (15)	192,2±10,2 (14)	305,0±14,6 (11)
	3	100,6±6,7 (9)	143,0±7,8 (9)	223,7±10,2 (9)
	4	87,3±2,8 (10)	125,4±3,8 (10)	154,1±5,8 (10)
120	1	116,6±5,4 (10)	217,4±5,2 (10)	305,8±19,8 (10)
	2	75,9±4,0 (12)	161,2±9,4 (12)	266,3±17,0 (12)
	3	89,3±4,1 (9)	121,5±5,2 (9)	170,8±6,8 (9)
	4	78,5±2,8 (9)	100,2±2,6 (9)	128,4±3,7 (9)

Legend. 1) Phenazepam, 2) 3-hydroxy derivative of phenazepam, 3) demethyldiazepam, 4) demethylsulazepam; number of animals in group (n) shown in parentheses.

The significance of differences between the values of DPCT, DCTC, and DTE in the different groups was assessed by Student's t test. The significance of the effect and significance of the strength of the effect were assessed by Fisher's F_f criterion, by comparing it with the standard value, namely F_{st} ($P < 0.05$-0.01).

The strength of the effect of the various factors (A_I — difference in chemical structure in the series: phenazepam, its 3-hydroxy derivative, demethyldiazepam, demethylsulazepam; A_{II} — the same in the series: diazepam, sulazepam, demethyldiazepam; A_{III} — the time of the experiment: 5, 15, 30, and 120 min) on the value of the minimal effective doses of metrazol was determined by the equation:

$$h_x^2 = \frac{D_x}{D_y}$$

with an error equal to:

$$m_{h_x}^2 = (1 - h_x^2)\left(\frac{a-1}{N-a}\right),$$

where D_x is the intergroup sum of the squares of the deviations, D_y the total sum of squares of deviations of the complex, a the number of gradations of the factor, and N the total number of variants (the volume of the dispersion complex).

EXPERIMENTAL RESULTS

In the group of control animals values of DPCT, DCTC, and DTE were 37.1 ± 1.46, 53.8 ± 2.59, and 82.6 ± 4.62 mg/kg, respectively. Determination of the analogous parameters in mice of the experimental group revealed the appearance of a significant ($P < 0.01$) anticonvulsant action as early as 5 min after injection of the test compounds into the animals, and this persisted throughout the experiment (Table 1). Injection of phenazepam and its 3-hydroxy derivative had the strongest action on mice. The anticonvulsant effect of demethylsulazepam and demethyldiazepam was comparable with results obtained previously on male CBA mice [3]. The drugs had their strongest action in the time interval from 15 to 30 min of the experiment.

It can be concluded from the results of dispersion analysis of the numerical data obtained during this investigation (Table 2) that the anticonvulsant action of 1,4-benzodiazepines in a dose of 5 mg/kg within this time interval is a dynamic parameter. Within the dispersion complex presented above, dependence of differences in the magnitude of the effect on differences in chemical structure of the drug was significant throughout the period of the experiment for all parameters of the paroxysmal seizure (Table 2).

TABLE 2. Results of One-Factor Dispersion Analysis of Change in Values of Parameters of Convulsion depending on Chemical Structure of Drug (A_I) and Time Factor (A_{III})

Minimal effective doses of metrazol	Parameter	A_I				A_{III}			
		5 min	15 min	30 min	120 min	1	2	3	4
DPCT	N	42	46	48	40	65	61	36	38
	h_x^2	0,510	0,521	0,617	0,238	0,238	0,400	0,462	0,76
	$m_{h_x}^2$	0,039	0,034	0,026	0,036	0,051	0,043	0,050	0,022
	F_f	13,2	15,3	23,6	16,5	4,67	9,36	9,17	33,32
	F_{st}	2,8—4,3	2,8—4,3	2,8—4,3	2,9—4,4	2,5—3,7	2,5—3,7	2.9—4,5	2,9—4.5
DCTC	N	42	46	47	40	65	60	36	38
	h_x^2	0,552	0.514	0,589	0,835	0,255	0,324	0,508	0,389
	$m_{h_x}^2$	0,035	0,035	0,029	0,014	0,049	0,049	0,046	0,054
	F_f	15,6	14.8	20,53	60,9	5,1	6,59	10,9	7,22
	F_{st}	2,9—4,3	2,8—4,3	2,8—4,3	2,9—4,4	2,5—3,7	2,9—4,5	2,9—4,5	2,9—4.5
DTE	N	42	43	43	40	63	55	36	38
	h_x^2	0,716	0,788	0,792	0,735	0,400	0,199	0,485	0,574
	$m_{h_x}^2$	0,022	0,016	0,016	0,022	0,041	0,064	0,048	0,037
	F_f	32,0	48,3	49,5	33,35	9,67	3,10	10,1	15,3
	F_{st}	2.8—4.3	2.8—4,3	2,9—4.4	4,3—7,9	2,5—3,7	2,6—3,7	2.9—4,5	2,9—4,5

Legend. 1) Phenazepam, 2) 3-hydroxy derivative of phenazepam, 3) demethyldiazepam, 4) demethylsulazepam.

TABLE 3. Results of One-Factor Dispersion Analysis of Changes in Parameters of Convulsion depending on Time Factor (A_{III}) and Chemical Structure of Drug (A_{II})

Minimal effective doses of metrazol	Parameter	A_{II}				A_{III}		
		5 min	15 min	30 min	120 min	5	6	7
DPCT	N	42	40	36	38	54	56	46
	h_x^2	0,530	0,430	0,047	0,276	0,347	0,368	0,523
	$m_{h_x}^2$	0,024	0,031	0,058	0,041	0,039	0,036	0,034
	F_f	21,9	13,9	0,83	6,69	8,85	10,1	15,34
	F_{st}	3,2—5,2	3,2—5,2	3,3—5,3	3,3—5,2	2,8—4,2	2,8—4,2	2,6—4,2
DCTC	N	42	40	36	38	54	56	46
	h_x^2	0,493	0,327	0,201	0,341	0,303	0,258	0,489
	$m_{h_x}^2$	0,026	0,036	0,048	0,038	0,042	0,043	0,036
	F_f	18,9	8,9	4,2	9,0	7,2	6,0	13,4
	F_{st}	3,2—5,2	3,2—5,2	3,3—5,3	3,3—5,2	2,8—4,2	2,8—4,2	2,8—4,3
DTE	N	42	40	36	38	54	56	46
	h_x^2	0,513	0,380	0,292	0,287	0,535	0,287	0,461
	$m_{h_x}^2$	0,025	0,033	0,043	0,041	0,028	0,042	0,038
	F_f	20,5	11,32	6,82	7,0	19,1	6,7	11,9
	F_{st}	3,2—5,2	3,2—5,2	3,2—5,2	3,3—5,3	2,9—4,2	2,8—4,2	2,8—4,3

Legend. 5) Diazepam, 6) sulazepam, 7) demethyldiazepam.

The results of the previous investigation [3] are represented by a similar dispersion complex (Table 3). The results of dispersion analysis of the anticonvulsant action of sulazepam and its metabolites also point to a significant relationship between differences in the values of DPCT, DCTC, and DTE and the time of the experiment and chemical structure of this group of test compounds.

It can be concluded from the doses of metrazol causing different phases of the convulsive process (Table 1) and also from the results of dispersion analysis of these values (Tables 2 and 3) that the most stable and demonstrative results were revealed by dispersion analysis of values of DTE. This criterion can be effectively used to evaluate the anticonvulsant activity of the 1,4-benzodiazepines with different chemical structure over a wide range of duration of the experiment (5-120 min).

The fact that dispersion analysis is highly effective as a means of assessing the results of a pharmacological experiment will be noted.

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MECHANISM OF SPECIES DIFFERENCES IN SENSITIVITY OF MONKEYS AND DOGS TO THE EMETIC ACTION OF VARIOUS DRUGS

V. I. Legeza, M. G. Shagoyan,
M. F. Kamynina, I. V. Marko'skaya,
and K. S. Martirosov

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KEY WORDS: mechanism of vomiting; neurotransmitters; monkeys; dogs.

Monkeys and dogs differ significantly in their sensitivity to emetics acting on the chemoreceptive trigger zone (CTZ) of the vomiting center [3]. Agonists of dopamine receptors do not cause vomiting in monkeys [15], whereas injection of apomorphine and other dopaminomimetics into dogs is accompanied by a marked vomiting reaction [14, 17]. Meanwhile certain drugs, such as cardiac glycosides, induce vomiting in both monkeys and dogs [15, 17].

The object of the present investigation was to study the mechanisms determining species differences in the responses of monkeys and dogs to the emetic action of various drugs.

EXPERIMENTAL METHOD

Experiments were carried out on male monkeys *Macaca mulatta* weighing 3.5-4.5 kg and on mongrel dogs of both sexes weighing 8-16 kg. The sensitivity of the experimental animals was determined to the emetic action of apomorphine, L-dihydroxyphenylalanine (L-dopa), adrenaline, clonidine, 5-hydroxytryptophan (5-HTP), and sodium salicylate. The ability of reserpine, mexamine (5-methoxytryptamine), trimeperidine, metoclopramide, and phentolamine to prevent the emetic effect of sodium salicylate also was studied. Reserpine was given 24 h, and the other drugs 30 min, before injection of the emetic. The doses of the drugs tested are given in Tables 1 and 2. Apomorphine and trimeperidine were injected subcutaneously, mexamine, metoclopramide, and phentolamine intramuscularly, and the remaining drugs intravenously. The numerical results were subjected to statistical analysis by Fisher's accurate method [2].

EXPERIMENTAL RESULTS

The experiments in which drugs selectively exciting different monoaminergic structures of CTZ were administered to monkeys and dogs (Table 1) showed that a vomiting response was observed in monkeys only to injection of 5-HTP, a specific agonist of serotonin receptors [1, 4]. Meanwhile dogs, unlike monkeys, were highly sensitive to the emetic action of apomorphine and L-dopa, substances exciting dopaminergic receptors of CTZ [11, 12], and of the α-adrenomimetic clonidine. Doses of 5-HTP whose action is accompanied by marked behavioral disturbances did not induce vomiting in the animals of this species.

The results suggest that CTZ in monkeys does not possess dopaminergic and adrenergic receptors, and in dogs it does not contain serotonin receptors. Meanwhile sodium salicylate, whose emetic action in the modern view [1] is mediated through the serotoninergic structures of CTZ, induces vomiting both in monkeys and in dogs (Table 2).

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