ANALYSIS OF THE STRUCTURE OF COMPONENTS OF THE CONVULSANT ACTION OF METRAZOL IN MICE RECEIVING SULAZEPAM AND ITS METABOLITES

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The relationship between minimal effective doses of pseudoclonic and clonico-tonic convulsions (DPCC, DCTC) and also of tonic extension (DTE), evoked by intravenous injection of metrazol into mice and the effect of the anticonvulsant action of sulazepam and its metabolites (diazepam, desmethyldiazepam, and oxazepam) were investigated. All the compounds tested were shown to increase the values of the minimal effective doses based on recorded indices of the seizure, and anticonvulsant activity reached a maximum 15 min after injection of desmethyldiazepam, 15-30 min after injection of sulazepam and oxazepam, and 5-30 min after injection of diazepam. Clear correlation was established between the minimal effective doses of the recorded indices of the seizure in the animals of the control group and it continued after injection of the drugs. It is postulated that sulazepam and its metabolites increase the minimal effective doses of metrazol for the recorded effect but do not change the general pattern of the seizure and do not affect dispersion of the dose–effect curves of metrazol.

KEY WORDS: sulazepam; metabolites of sulazepam; metrazol seizures; regression equations.

A single injection of small doses of compounds of the 1,4-benzdiazepine series abolishes all components of the seizure evoked by metrazole (pseudoclonic convulsions, clonico-tonic convulsions, and clonic extension). Their ability to prevent the development of clonico-tonic convulsions is of great interest because some workers [9, 10] consider that this component corresponds to the petit mal seizure in man. In addition, the antimetrazol effect is regarded as an indicator reflecting the psychosedative action of drugs [5, 8].

The object of this investigation was to compare the role of sulazepam and its metabolites in the formation of the seizure induced in mice by administration of metrazol.

EXPERIMENTAL METHOD

The writers showed previously that sulazepam (7-chloro-1-methyl-5-phenyl-1,3-dihydro-3H-1,4-benzdiazepine-2-thione) in vivo in experimental animals undergoes enzymic desulfonation, demethylation, and ^{3}C-hydroxylation [3, 4], with the formation of basic metabolites: diazepam, desmethyldiazepam, and oxazepam. These compounds were synthesized in the writers' laboratory and used for the present investigations.

Experiments were carried out on 266 male CBA mice weighing 18-22 g. The animals were given an intraperitoneal injection of sulazepam, diazepam, desmethyldiazepam, and oxazepam (5 mg/kg) as an aqueous emulsion with Tween-80. Control animals received the emulsion only. Minimal doses causing pseudoclonic convulsions (DPCC), clonico-tonic convulsions (DCTC), and clonic extension (DTE) in response to injection of 1% metrazol solution into the mice at the rate of 0.01 ml/min were established 5, 15, 30, 120, and 240 min after injection of the test compounds.

The paired dose values were subjected to statistical analysis [7].

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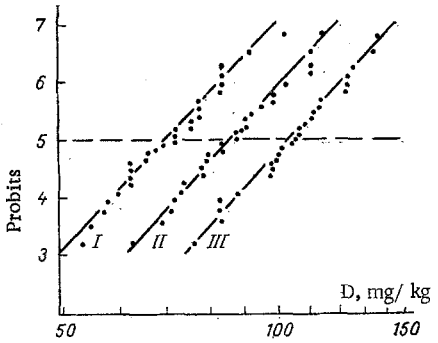

Fig. 1. Effect (in probits) as a function of dose of metrazol for group of control animals: I) DPCC; II) DCTC; III) DTE.

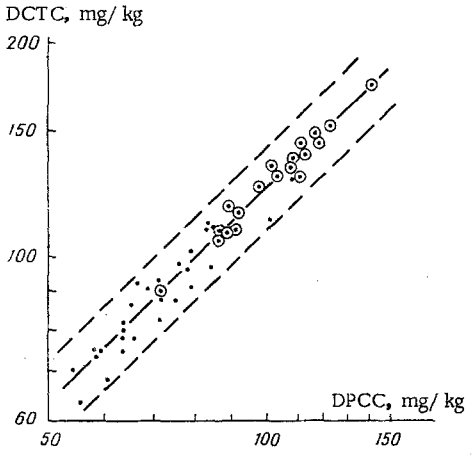

Fig. 2

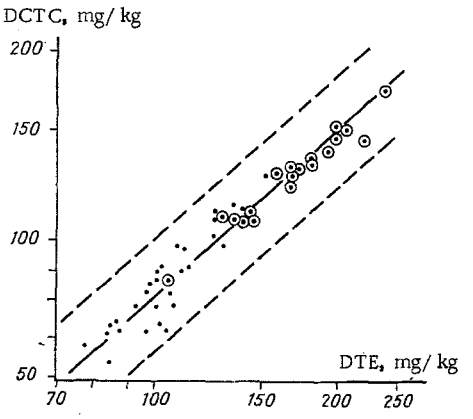

Fig. 3

Fig. 2. Correlation between DCTC and DPCC. Points show values of single experiments in control series, circles show mean values of minimal effective doses after injection of sulazepam and its metabolites, broken lines represent confidence limits of regression line.

Fig. 3. Correlation between DTE and DCTC. Legend as in Fig. 2.

EXPERIMENTAL RESULTS AND DISCUSSION

Investigation of the distribution of the minimal effective doses in the control animals showed (Fig. 1) that the experimental data obey ($P < 0.01$) the linear relationship between effect and logarithm of dose classically observed in pharmacology [6]:

$$E = a \log D + b, \tag{1}$$

where E is the effect in probits and D the dose of the biologically active substance. Corresponding regression equations for the recorded indices of the seizure, found by the method of least squares [7], had the following form:

$$E = 13.64 \lg_{\text{DPCC}} - 20.2, \tag{2}$$

$$E = 13.56 \lg_{\text{DCTC}} - 21.4, \tag{3}$$

$$E = 13.58 \lg_{\text{DTE}} - 22.5. \tag{4}$$

TABLE 1. Changes in Minimal Values of DPCC, DCTC, and DTE with Time after Injection of Sulazepam and its Metabolites into Animals in a Dose of 5 mg/kg (M ± m)

Drug	Number of animals	Time, min	DPCC	DCTC	DTE
Control	29	—	71,57±2,63	87,83±3,14	107,05±3,48
Sulazepam	15	5	85,61±2,43	107,91±2,81	142,72±4,37
	14	15	103,91±4,49	131,99±6,88	169,13±9,34
	13	30	110,83±4,80	129,49±5,40	164,71±5,64
	14	120	89,19±4,44	109,39±3,91	135,40±6,14
	12	240	90,81±3,47	109,57±4,06	139,69±7,2
Diazepam	14	5	110,84±3,11	139,82±4,5	193,43±5,33
	13	15	113,99±4,57	149,45±8,06	198,43±9,81
	13	30	116,8±5,1	148,46±5,8	193,64±6,36
	14	120	91,71±3,07	115,79±3,19	144,67±3.86
Desmethyldiazepam	13	5	101,53±3,13	134,74±5,51	183,76±10,33
	13	15	141,35±6,9	176,55±8,52	235,88±11,96
	10	30	108,53±4,6	134,72±4,8	181,70±5,95
	10	120	108,11±3,85	131,39±3,99	163,09±5,39
	17	240	86,01±2,36	104,65±3,73	113,09±3,06
Oxazepam	15	5	88,87±3,76	120,75±4,9	164,93±8,48
	12	15	111,53±5,96	142,73±5,07	223,84±12,8
	11	30	122,34±6,84	153,63±5,72	197,95±9,73
	14	120	97,12±4,67	127,24±5,68	159,9±6,02

In the comparative study of experimental data in pharmacology an essential part is played by the establishment of parallelism between the straight lines of dose versus effect when comparing doses producing the effect in 50% of objects tested (ED_{50}). In this investigation there was no need to use the method of probit analysis [2] or to compare the corresponding regression coefficients [1] for this purpose, for it follows from equation (1) that the logarithms of the minimal effective doses producing the recorded effects obeyed the law of the normal distribution. In that case ED_{50} coincided with the geometric mean of the population and its value was 70.4 (66.8-74.2) mg/kg for DPCC, 86.6 (82.1-91.3) mg/kg for DCTC, and 105.5 (100.6-111.0) mg/kg for DTE.

Both in the control and after injection of sulazepam and its metabolites the values of the arithmetic means of the populations of minimal recorded doses were not significantly higher ($P > 0.05$) than those of the corresponding geometric means. It follows from the values of the confidence limits for these effective doses that the values of the free terms of the regression equations (2) and (3), and (3) and (4), differed significantly ($P < 0.05$).

Fisher's criterion [7] can serve to reflect ($P < 0.05$) the absence of differences between the slope of the dose-effect lines, for the dispersions of distribution of the logarithms of the minimal effective doses reflects not only the range, but also the specific pattern of their distribution. No significant differences ($P > 0.05$) were found for the dispersions of logarithms of the doses of the recorded effects. Consequently, the coefficients for equations (1), (2), and (3) did not differ significantly. Further investigations showed that the values of DPCC, DCTC, and DTE in the individual experiments (paired values) in the animals of the control group correlated strongly ($P < 0.01$). The coefficients of correlation were 0.95 for DPCC and DCTC, 0.83 for DTE and DPCC, and 0.92 for DTE and DCTC.

The relationship between the recorded components of the seizure can be expressed as follows:

$$\lg \text{DPCC} = 0.867 \lg \text{DTE} - 0.097, \quad (5)$$

$$\lg \text{DCTC} = 0.976 \lg \text{DTE} - 0.061, \quad (6)$$

$$\lg \text{DPCC} = 1.000 \lg \text{DCTC} - 0.032. \quad (7)$$

Since these equations represent linear functions in a logarithmic system of coordinates, dispersions of logarithms of the doses for the corresponding effects (Fig. 2 and 3) were used to calculate the confidence limits for the regression:

ΔDPCC = $\pm$ log 16.11 and ΔDTE = $\pm$ log 22.55 for equation (5),
ΔDCTC = $\pm$ log 13.7 and ΔDTE = $\pm$ log 15.4 for equation (6),
Δ DPCC = $\pm$ log 8.66 and ΔDCTC = $\pm$ log 10.79 for equation (7).

Administration of sulazepam and its metabolites to the experimental animals led to a rapid rise in DPCC, DCTC, and DTE. For instance, 15 min after their injection, all the preparations tested had a significant ($P < 0.01$) anticonvulsant action (Table 1).

The anticonvulsant activity, with respect to all indices recorded, reached a maximum (Table 1) for sulazepam and oxazepam between 15 and 30 min, for desmethyldiazepam after 15 min, and for diazepam 5 and 30 min. A further increase in the duration of the experiment led to a reduction in the effect. Only recorded indices exceeding the corresponding control values with a significance of $P < 0.01$ are considered (and given in Table 1) in this paper.

Comparison of the anticonvulsant action of the compounds revealed the following fact: Desmethyldiazepam had the strongest anticonvulsant activity, sulazepam had weakest anticonvulsant activity, and diazepam and oxazepam occupied an intermediate position for ability to increase minimal effective doses of metrazol (Table 1).

Comparison of the minimal effective doses of the recorded components of the seizure in animals of the control group with the values of the corresponding effects formed after administration of sulazepam and its metabolites to the animals shows that the values of DPCC, DCTC, and DTE in the latter were higher, but the general structure of the convulsions was the same (compare Figs. 2 and 3). The confidence limits of the corresponding regression equations for the values of DPCC, DCTC, and DTE (compare Figs. 2 and 3) obtained after injection of sulazepam and its metabolites at all times of the investigation were never transgressed.

No significant differences likewise were found in the dispersions of the logarithms of the minimal doses of all indices of the seizure recorded, either in the control or at all times after injection of sulazepam and its metabolites.

It follows from what has been said that sulazepam and its metabolites increase the values of DTCC, DCTC, and DTE, but have no effect on the general pattern of the seizure – on interaction between its components or the specific nature of dispersion of the dose–effect curves.

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