# THE COMPARATIVE ANTICONVULSANT ACTIVITY OF PHENOTHIAZINE DERIVATIVES IN EXPERIMENTAL ELECTRICAL SHOCK

K. S. Raevskii

Division for Determination of the Physiological Activity of New Products of Chemical Synthesis (Head – Candidate in Medical Sciences Yu. I. Vikhlyaev), AMN SSSR Institute of Pharmacology and Chemotherapy (Dir. – Active Member AMN SSSR V. V. Zakusov), Moscow
Department of Pharmacology (Head – Active Member AMN SSSR V. M. Karasik), Leningrad Institute of Pediatric Medicine
(Presented by Active Member AMN SSSR V. V. Zakusov)
Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 2, pp. 64-68, February, 1961
Original article submitted December 22, 1959

The question of the anticonvulsant properties of Aminazine and other phenothiazine derivatives has interested many researchers; the answers to this question, however, are still incomplete and, to some extent, contradictory. This is particularly true of works in which electrical shock has been used to reproduce a convulsive attack. For example, Hauschild [7], Tanaka and Kawasaki [12] and also Haas [6] found that chlorpromazine and certain other phenothiazine derivatives possess anticonvulsant activity. Conversely, other authors have shown chlorpromazine to be ineffective for convulsions of this type [4, 9]. The clinical and experimental data recently published on the so-called convulsant effect of chlorpromazine [2, 14] have revived interest in this question. Its practical significance is obvious due to the fact that Aminazine and other phenothiazine derivatives are often used in psychiatry to treat diseases of a convulsive nature [3].

The purpose of this work was to study Aminazine and other phenothiazine derivatives as to their ability to alter the course of a convulsive attack induced by electrical shock and to compare them in this respect with certain antiepileptics – Luminal and Diphenin [5,5-diphenylhydantoin sodium].

## EXPERIMENTAL METHOD

We used the method of electrical shock* [13], modified for white mice [11] to judge the anticonvulsant action of the substances. Fifty ma alternating current was passed through the animal's head with a frequency of 50 cps and stimulation duration of 0.2 second with the aid of corneal electrodes. The convulsive attack developed rapidly; a long phase of tonic extension of the posterior extremities dominated the picture of the attack, later giving way to a phase of clonic convulsions. Absence of the tonic extension phase of the attack served as a criterion of the anticonvulsant effect. The central relaxation of the skeletal musculature characterizing the pharmacological effect of neuroplegic substances was regarded as a side effect secondary to the anticonvulsant effect and was determined by the "revolving roller" method [5]. The same method was used to study the side effects of Luminal and Diphenin, which in toxic doses induce ataxia, unsteady gait and disturbances of equilibrium and motor coordination [10]. The results of the experiments were compared. The median effective doses, or $ED_{50}$, required to produce the anticonvulsant effect and the $TD_{50}$ required to evoke the side effects were determined by the method of Litchfield and Wilcoxon [8]. The $TD_{50}$ was divided by the $ED_{50}$ to obtain the "protective index" characterizing the therapeutic range of the preparations. D = 0.05 was used to

* Termed M. E. S. test in the English literature.

compute the authentic amounts of the $ED_{50}$ and $TD_{50}$. The time at which the maximal anticonvulsant effect, or the peak of the effect, developed and the over-all duration of the effect were determined for each substance. This was done as follows: the experimental substance was administered to two groups of five mice each in doses equal to the $ED_{50}$; electrical stimulation was then administered by the method of electrical shock after 30 minutes to the first group of mice and after one hour to the second group. This procedure was repeated hourly until the complete disappearance of the anticonvulsant effect. In this way, the time at which the anticonvulsant effect developed, the time the effect became maximal, or the peak of the effect, and the total duration of the effect were determined. All the substances were analyzed by the electrical shock test according to the peak time of their anticonvulsant effects. The experimental animals used were male and female white mice weighing 24-29 g each.

We tested the following compounds: 10-(3-dimethylaminopropyl)-2-chlorphenothiazine hydrochloride (Aminazine), 10-(3-dimethylaminopropyl)-phenothiazine hydrochloride (Propazine), 10-(3-dimethylaminopropyl)-2-acetylphenothiazine maleate (Acepromazine), 10-(N-methylpiperidyl-3-methyl) phenothiazine acetate (Mepazine), 10-(N-methylpiperidyl-3-methyl)-2-chlorphenothiazine acetate (chlorine-containing Mepazine analog), 10-(2-diethylaminoethyl) phenothiazine hydrochloride (Dinezine), 10-(2-diethylaminoethyl)-2-chlorphenothiazine (chlorine-containing Dinezine analog), 10-[3-(I-β-hydroxyethylpiperazinyl-4) propyl]-2-chlorphenothiazine tartrate (Étaperazine), 10-[3-(I-methyl-4-piperazinyl)-propyl]-2-chlorphenothiazine hydrochloride (Compazine), 10-(β-diethylaminoproponyl)-2-chlorphenothiazine hydrochloride (Chloracizin) and the anticonvulsant substances Luminal (sodium salt) and Diphenin (5,5-diphenylhydantoin sodium). All the phenothiazine derivatives were intraperitoneally injected in aqueous solutions; Luminal was injected subcutaneously, and Diphenin was perorally administered through a probe in a 1% starch solution.

## EXPERIMENTAL RESULTS

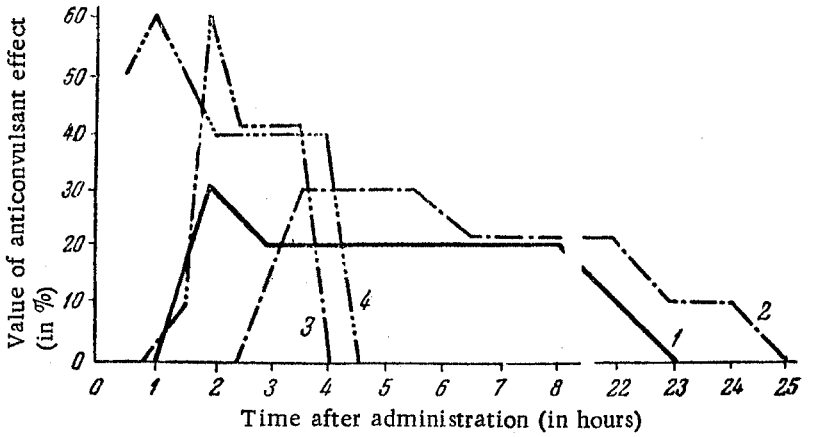
All ten of the experimental phenothiazine derivatives were found to be active anticonvulsants. The administration of these substances altered the typical course of the convulsive attack induced in white mice by electrical stimulation. The tonic extension phase, which was normally predominant in the picture of these attacks, was not observed, and the attack became clonic in character. Luminal and Diphenin manifested a similar anticonvulsant effect. For Aminazine, Propazine, Acepromazine and Mepazine, the $ED_{50}$ was about the same (around 35 mg/kg); Aminazine and Acepromazine, however, caused such acute relaxation of the skeletal musculature that the animals became unable to move and acutely depressed, reacting weakly to external stimulations. The use of Mepazine in the same doses did not cause significant disturbances in the gait, posture or behavior of the animals. This indicates that the therapeutic value of a potential anticonvulsant agent cannot be defined by its $ED_{50}$ alone; some criterion of the side effects, for which we used the $TD_{50}$, must be included in such calculations. The ratio $TD_{50}/ED_{50}$ characterizes the therapeutic range of a substance's effect and is defined by the term "protective index." Other important criteria of an anticonvulsant substance's value are the speed with which its effect develops and the total duration of the effect. All these values are given in the table.

All the experimental phenothiazine derivatives can be divided into three groups of anticonvulsant activity: 1) the most active – Aminazine, Propazine, Acepromazine and Mepazine; 2) less active – Chloracizin, Dinezine and the chlorine-containing analogs of Dinezine and Mepazine; and 3) the least active – Étaperazine and Compazine. The picture is different, however, if the protective index rather than the $ED_{50}$ is used as the criterion for comparative evaluation. The last column in the table shows only four compounds among the phenothiazine derivatives to have an index of more than unity, and of these compounds, only Mepazine and the chlorine-containing Dinezine analog possess a substantial therapeutic range. The protective indices of Propazine, Dinezine and the chlorine-containing Mepazine analog are close to unity, while those of Aminazine, Acepromazine, Étaperazine and Compazine are several times less than unity. According to their protective indices, Aminazine and Mepazine are not of the same value as potential anticonvulsant agents. Mepazine is more than 11 times as strong as Aminazine in this respect.

Another important criterion of the value of an anticonvulsant agent is the duration of its effect after a single administration. The graph shows these data for Aminazine, Mepazine, Luminal and Diphenin. We see that the effect of the phenothiazine derivatives is characterized by comparatively early manifestation, rapid attainment of its peak and a short total duration. The effect of Aminazine was less lasting than that of Mepazine. The chlorine-containing analogs of Mepazine and Dinezine produced the most lasting effects of the other penothiazine derivatives.

Anticonvulsant and Side Effects of Certain Phenothiazine Derivatives, Luminal and Diphenin

| Substance | Chemical structure | | Anticonvulsant activity as per electrical shock test | | | Side effect as per revolving roller test | Protective index |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $ED_{50}$ (D = 0.05 used to compute authentic range) | peak of effect (in hours) | total duration of effect (hr) | $TD_{50}$ (D = 0.05 used to compute authentic range) | $P.I.=\frac{TD_{50}}{ED_{50}}$ |
| Aminazine | $-(CH_2)_3-N-(CH_3)_2$ | Cl | 34 (30.6—37.8) | 2 | 2 | 6.4 (5.4—7.6) | 0.2 |
| Propazine | $-(CH_2)_3-N-(CH_3)_2$ | H | 32.8 (31.0—34.8) | 1.5 | 2 | 40 (35.4—45.2) | 1.2 |
| Acepromazine | $-(CH_2)_3-N-(CH_3)_2$ | $COCH_3$ | 32.5 (28.0—37.8) | 0.5 | 1.5 | 3.9 (3.0—5.0) | 0.1 |
| Mepazine |  | H | 35 (31.8—38.5) | 1 | 3.5 | 82 (74—91) | 2.3 |
| Chlorine-containing Mepazine analog |  | Cl | 79.0 (71.1—87.6) | 1.5 | 4 | 77.0 (68.1—87.0) | 0.99 |
| Dinezine | $-(CH_2)_2-N-(C_2H_5)_2$ | H | 66.2 (58.6—74.9) | 0.5 | 0.5 | 76.0 (59.4—97.3) | 1.1 |
| Chlorine-containing Dinezine analog | $-(CH_2)_2-N-(C_2H_5)_2$ | Cl | 70.0 (65.4—74.9) | 2 | 3.5 | 102.5 (93.3—112.9) | 1.5 |
| Etaperazine |  | Cl | 100.0 (91.8—108.6) | 2 | 1.5 | 9.0 (8.2—9.9) | 0.1 |
| Compazine |  | Cl | 114.0 (106.6—122.0) | 1 | 1 | 18.6 (16.0—21.6) | 0.16 |
| Chloracizin | $-CO-(CH_2)_2-N-(CH_3)_2$ | Cl | 56.0 (49.1—63.8) | 0.5 | 2.5 | — | — |
| Luminal-Na | 5-ethyl-5'-phenylbarbiturate (Na salt) | | 36.8 (33.2—40.8) | 2 | 20 | 98.0 (86.8—110.6) | 2.7 |
| Diphenin | 5,5'-diphenylhydantoin | | 20.0 (17.4—23.0) | 3.5 | 20.5 | 358.0 (317.0—405.0) | 18.0 |




Duration and peak of anticonvulsant effect of neuroplegic and antiepileptic substances. 1) Luminal; 2) Diphenin; 3) Aminazine; 4) Mepazine.

It was interesting to compare the anticonvulsant properties and side effects of the phenothiazine derivatives with those of Luminal and Diphenin. The data for the latter two substances can be found at the bottom of the table. When the phenothiazine derivatives with the most active anticonvulsant effect were compared with these typical antiepileptic agents, Aminazine, Propazine, Acepromazine and Mepazine were found to be just as active as Luminal, although their effect was less durable; only Mepazine approximated Luminal, however, in value of protective index. Further, both Mepazine and Luminal were considerably less active than Diphenin.

As to the relationship between the chemical structure and pharmacological effect of the experimental substances, it is evident that the anticonvulsant activity of three compounds having carbon in the second position of the phenothiazine ring, but with different $R_2$ radicals (Propazine, Aminazine and Acepromazine), is approximately the same, while the central relaxant effect of the chlorine-containing analog (Aminazine) is much stronger than that of the nonsubstituted compound (Propazine), and the same effect is stronger still in the case of the acetyl analog (Acepromazine). Other effects (adrenolytic effect, potentialization of narcotic substances) of these substances are known to be strengthened by the substitution of a chlorine atom or acetyl radical [1] for the hydrogen atom, with carbon in the second position. It was also demonstrated in [1] that the ability of Mepazine to potentialize thiopental sleep is approximately half as great as that of its analog, although, as our table shows, the anticonvulsant effect of Mepazine is about twice as strong as that of its analog. On the basis of these data, one can propose that the anticonvulsant effect of neuroplegic substances should be differentiated from their general "central" effect. The mechanism of this effect, however, has not yet been sufficiently investigated.

## SUMMARY

Ten phenothiazine derivatives were tested on mice for anticonvulsant potency at the time of their peak effect by the maximal electroshock seizure test (M. E. S.). Two clinically employed antiepileptic drugs – Luminal and diphenylhydantoin – were studied by the same technique. Their side effect (neurological toxicity) was investigated by the "rolling roller" method. All substances were found to abolish the tonic extensor phase of the maximal seizure. $ED_{50}$, $TD_{50}$ and protective indices (P. I.) for all drugs have been determined. Diphenylhydantoin, Luminal and Mepazine had the highest P. I.; protective indices of Propazine, Dinezine, chlorsubstituted analogs of Mepazine and Dinezine approached 1. Aminazine, Acepromazine, Étaperazine and Compazine were effective only in toxic doses. The suggestion is made that anticonvulsive action of phenothiazine derivatives differed from their general "central" effect.

## LITERATURE CITED

1. Yu. I. Vikhlyaev, in: New Data on the Pharmacology and Clinical Use of Phenothiazine Derivatives [in Russian] (Moscow, 1958) p. 27.
2. N. N. Zak, Zhur. Nevropatol. i Psikhiatr. 57, 6, 750 (1957).
3. Iv. Temkov, et al., Zhur. Nevropatol. i Psikhiatr. 58, 10, 1164 (1958).

4. A. Balestrieri, Arch. Int. Pharmacodyn. 100, 361 (1955).
5. N. W. Dunham and T. S. Miya, J. Am. Pharm. Ass., Sci. Ed. 46, 208 (1957).
6. H. Haas, Arzneimittel-Forsch 8, 20 (1958).
7. F. Hauschild, quoted from: K. Feller, Arch. exp. Path. u. Pharmak. 225, 90 (1955).
8. J. T. Litchfield and F. Wilcoxon, J. Pharmacol. and Exp. Ther. 96, 99 (1949).
9. O. Nieschulz, K. Popendiker, and I. Hoffman, Arzneimittel-Forsch. 5, 680 (1955).
10. E. A. Swinyard, J. Am. Pharm. Ass., Sci. Ed. 38, 201 (1949).
11. E. A. Swinyard, W. C. Brown, and L. S. Goodman, J. Pharmacol. and Exp. Ther. 106, 319 (1952).
12. K. Tanaka and Y. Kawasaki, Jap. J. Pharmacol. 6, 115 (1957).
13. J. E. P. Toman, E. A. Swinyard, and L. S. Goodman, J. Neurophysiol. 9, 231 (1946).
14. D. H. Tedeschi, et al., J. Pharmacol. and Exp. Ther. 123, 35 (1958).