Anticonvulsant Properties of Some N-Substituted Hydantoins

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The neurotoxic doses, anticonvulsant potencies, and protective indexes of three N-substituted hydantoins were compared with similar values concomitantly determined for phenantoin and diphenylhydantoin. The experimental agents induced overt evidence of neurotoxicity only after doses which were significantly higher than those required for phenantoin and diphenylhydantoin and exhibited anticonvulsant potencies which were generally less than those for the two clinical agents. Of the experimental compounds, 1-methyl-5,5-phenylethylhydantoin appeared most promising. Structure-activity studies indicated that mono-methyl substitution on a nitrogen of the hydantoin ring exerted a more favorable influence on anticonvulsant activity than either di-methyl or mono-ethyl substitution and that optimal activity was obtained when the methyl substitution is on the nitrogen in position 3 of the hydantoin ring.

THE AVAILABILITY of three N-substituted hydantoins offered the opportunity to explore further the structure-activity relation of some phenantoin congeners. Our interest in these compounds was also stimulated by a preliminary clinical report (1) which indicated that one of them is clinically effective in epilepsy. For this reason it seemed desirable to obtain precise experimental data on this agent in order to provide additional information on the correlation between the laboratory assay and clinical efficacy of anticonvulsant drugs.

METHODS

Male albino mice (Carworth Farms, CF No. 1 strain) were used as experimental animals. They were maintained on Rockland mouse diet and allowed free access to food and water except for the short time they were removed from their cages for testing. The following anticonvulsant agents were studied: 1-methyl-5,5-phenylethylhydantoin (No. 1),[1] 1,3-dimethyl-5,5-phenylethylhydantoin (No. 2),[1] 3-ethyl-5,5-phenylethylhydantoin (No. 3),[1] 3-methyl-5,5-phenylethylhydantoin (phenantoin),[2] and diphenylhydantoin.[3] All drugs were given orally as suspensions in 6% acacia solution, except for diphenylhydantoin which was given as the sodium salt in an aqueous solution. The concentration of the drug suspension or solution employed was such that the dose administered always represented 1 ml. per 100 Gm. body weight.

Anticonvulsant potencies (ED_{50}s) were determined by three tests (two electrical and one chemical). The tests based on electrically-induced convulsions measured the ability of a drug to prevent the hindleg tonic-extensor component of maximal electroshock seizures evoked by supramaximal current (MES test; 50 ma. alternating current, 0.2-second stimulus duration, corneal electrodes) and to elevate the threshold for low-frequency electroshock seizures (l.f. EST) induced in mice by unidirectional current delivered at an intensity twice threshold (0.2-millisecond duration, 3-second stimulus duration, six pulses per second). The test based on chemically-induced convulsions measured the ability of a drug to afford complete protection against seizures induced by the subcutaneous injection of pentylenetetrazol (Metrazol; 85 mg./Kg.; s.c. Met. test). A Grass stimulator (model S4B) was used for the l.f. EST test; otherwise the details of the various procedures, the end points employed in mice, and the characteristics of the electroshock apparatus have been described elsewhere (2–4). In addition, the mean neurotoxic dose (TD_{50}) was determined for each drug. The end point for minimal neurotoxicity was muscular incoordination, based on the inability of the animal to remain for 1 minute on a horizontal rod rotating at 6 r.p.m. Each drug was tested at the time of its peak activity as measured by the neurotoxicity test. For the determination of the ED_{50} or TD_{50}, groups of 8 to 12 mice were given various doses of drug until at least three points were established in the range between 0 and 100% seizure protection or minimal neurotoxicity. The results obtained were then plotted on logarithmic probability paper and a regression line was fitted to the plotted points by eye. From this plot of the data the respective ED_{50}, TD_{50}, 95% fiducial limits, and protective index (P.I. = TD_{50}/ED_{50}) were calculated by the method of Litchfield and Wilcoxon (5).

RESULTS

The time of peak effect, neurotoxicity, and anticonvulsant potency of the N-substituted compounds in mice, in comparison with phenantoin and diphenylhydantoin, are shown in Table I. The time of peak effect as measured by the test for minimal neurotoxicity varies from 1 hour for No. 3 to 3

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[1] Kindly supplied by Mr. Harry Althouse, Sandoz Pharmaceuticals. Sandoz code numbers: No. 1 (N-3), No. 2 (N-5), and No. 3 (N-7).
[2] Marketed as Mesantoin by Sandoz.
[3] Marketed as Dilantin by Parke Davis.

TABLE I.—ANTICONVULSANT POTENCY, NEUROTOXICITY, AND PROTECTIVE INDEX (P.I.) OF SOME N-SUBSTITUTED HYDANTOINS AND DIPHENYLHYDANTOIN IN MICE[a]

Drug	Time Peak Effect, min.[b]	TD_{50}	Assay: MES ED_{50}	MES P.I.	l.f. EST ED_{50}	l.f. EST P.I.	s.c. Met. ED_{50}	s.c. Met. P.I.
No. 1	180	292	102	2.86	75	3.89	75	3.89
		(274–311)	(90–115)	(2.60–3.15)	(63–89)	(3.22–4.71)	(60–94)	(3.09–4.90)
No. 2	150	275	110	2.50	100	2.75	83	3.31
		(229–330)	(101–120)	(2.05–3.05)	(86–116)	(2.18–3.47)	(63–110)	(2.40–4.57)
No. 3	60	442	230	1.92	175	2.53	265	1.67
		(402–486)	(172–308)	(1.40–2.63)	(141–217)	(1.96–3.26)	(215–326)	(1.33–2.10)
Phenantoin	90	200	46	4.35	65	3.08	40	5.00
		(167–240)	(39–53)	(3.45–5.48)	(49–87)	(2.20–4.31)	(34–48)	(3.91–6.40)
Diphenyl-hydantoin	180	110	16	6.88	61	1.80	[c]	[c]
		(88–138)	(13–19)	(5.13–9.22)	(41–92)	(1.14–2.84)		

[a] Values in parentheses are 95% fiducial limits; ED_{50}s in mg./Kg. [b] By neurotoxicity test. [c] Not effective by this test.

hours for No. 1 and diphenylhydantoin. Phenantoin and No. 2 exhibit peak activity at $1^1/_2$ and $2^1/_2$ hours, respectively. The TD_{50}s range from 110 mg./Kg. for diphenylhydantoin to 442 mg./Kg. for No. 3. Phenantoin, No. 2, and No. 1 induce overt symptoms of neurotoxicity in 50% of the animals after doses of 200, 275, and 292 mg./Kg., respectively.

With regard to anticonvulsant potency, Table I shows that, except for No. 1 by the l.f. EST test and for diphenylhydantoin by the s.c. Met. test, the N-substituted hydantoins are considerably less potent than either phenantoin or diphenylhydantoin. This is clearly established by examining the potency ratios obtained by dividing the ED_{50} for phenantoin by the ED_{50} for No. 1. Thus, the potency ratio is 0.45 by the MES test and 0.53 by the s.c. Met. test. On the other hand, there is no significant difference between the potency of No. 1 and the two clinically established hydantoins by the l.f. EST test. Furthermore, No. 1 is effective by the s.c. Met. test in a dose of 75 mg./Kg., whereas diphenylhydantoin is ineffective by this test.

Table I also lists the protective indexes (P.I.) of the five compounds as derived from their TD_{50}s and ED_{50}s by the various tests. It may be seen from the table that No. 1 exhibits the highest P.I. of the experimental N-substituted compounds by all three tests and that these P.I.s are significantly higher than those for No. 3. However, the difference between the P.I.s for No. 1 and No. 2 is significant only in the case of the l.f. EST test. Except for No. 1 by the l.f. EST test, the P.I.s for the N-substituted compounds are all less than those for phenantoin. Thus, the P.I.s for No. 1 and phenantoin by the MES test are 2.86 and 4.35, respectively, and by the s.c. Met. test 3.89 and 5.00, respectively. Although the N-substituted compounds all have P.I.s less than diphenylhydantoin by the MES test, they all exhibit P.I.s greater than those for diphenylhydantoin by the l.f. EST and s.c. Met. tests. In addition, No. 1 has the highest P.I. of any of the five compounds examined when compared on the basis of the l.f. EST test; however, the difference between the P.I.s for No. 1 and phenantoin by this test is not significant.

DISCUSSION

The data presented indicate that, except for diphenylhydantoin as measured by the s.c. Met. test, the profiles of anticonvulsant activity of the N-substituted compounds are qualitatively similar to those of phenantoin and diphenylhydantoin. Thus, all drugs tested have the ability to abolish the hindleg tonic-extensor component of maximal electroshock seizures and to elevate the threshold for l.f. EST and s.c. Met. in nontoxic doses. The ineffectiveness of diphenylhydantoin by the s.c. Met. test is in agreement with other reports from our laboratories (3, 6, 7).

The sequence of neurotoxic manifestations which follow the oral administration of the experimental N-substituted hydantoins and phenantoin is very similar, but there is a threefold difference in the time for maximum neurotoxic effect (compare No. 1 and No. 3, Table I) and a somewhat more than twofold difference in the dosage required to produce minimal neurotoxicity (compare No. 3 and phenantoin, Table I). The ratios of the TD_{50}s (phenantoin or diphenylhydantoin/N-compounds) indicate that the N-substituted compounds are only 45 to 73% as toxic as phenantoin and 25 to 40% as toxic as diphenylhydantoin.

With regard to anticonvulsant activity, No. 1 is more effective in elevating the threshold for electrically- and chemically-induced seizures than in modifying the pattern of seizures induced by supramaximal electroshock. Conversely, diphenylhydantoin is more effective in modifying the pattern of maximal electroshock seizures than in elevating the threshold for electrically- and chemically-induced seizures. On the other hand, No. 2, No. 3, and phenantoin do not exhibit significant selectivity of action when compared for ability to modify pattern and to elevate seizure threshold.

The two methyl-substituted hydantoins (No. 1 and No. 2) are significantly more potent by all three tests and significantly more toxic than the ethyl-substituted hydantoin (No. 3). Except for No. 1 by the l.f. EST test, there is no significant difference in the anticonvulsant potency or the neurotoxicity of No. 1 and No. 2. A comparison of phenantoin and No. 2 reveals that mono-methyl substitution (phenantoin) has more influence on anticonvulsant potency than di-methyl substitution (No. 2). Comparison of the anticonvulsant potencies of the two mono-methyl compounds (No. 1 and phenantoin) indicates that methyl substitution in position 3 induces approximately a twofold increase in the anti-MES and anti-s.c. Met. activity, but has no significant effect on the l.f. EST. These observations suggest that N-methyl substitution increases anticonvulsant activity more than does N-

ethyl substitution, and that for optimal activity the N-methyl substitution on the hydantoin ring should be in position 3, as in phenantoin.

An evaluation of the P.I.s derived from the experimental data reveals that No. 1 has the highest value of the three experimental drugs tested. Comparison of the P.I.s of No. 1 and those of the two clinical compounds indicates that, except for diphenylhydantoin by the s.c. Met. test, all three agents exhibit satisfactory P.I.s by all three tests. The difference between the P.I.s for No. 1 and phenantoin by the l.f. EST and s.c. Met. tests is not significant.

A preliminary clinical report (1) and unpublished observations communicated to us by Dr. Leonard W. Jarcho, Division of Neurology, University of Utah, suggest that No. 1 is effective in some patients with grand mal epilepsy resistant to other anticonvulsant therapy and, in contrast to phenantoin and diphenylhydantoin, in some patients with petit mal. Observed side effects are said not to be serious and, to date, gum hypertrophy has not been reported. Should more extensive clinical studies unequivocally establish that No. 1 is effective in patients not responding to conventional antiepileptic therapy and does indeed possess a broader spectrum of activity and lower incidence of toxicity than do the related hydantoins, it would represent a significant addition to the drugs currently available for the treatment of epilepsy.

SUMMARY

The anticonvulsant potencies (ED_{50}s) of three experimental N-substituted hydantoins, 1-methyl-5,5-phenylethylhydantoin (No. 1), 1,3-dimethyl-5,5-phenylethylhydantoin (No. 2), and 3-ethyl-5,5-phenylethylhydantoin (No. 3), and of phenantoin and diphenylhydantoin were determined in mice by the following three tests: maximal electroshock seizure pattern (MES) test, low-frequency electroshock seizure threshold (l.f. EST) test, and pentylenetetrazol seizure threshold (s.c. Met.) test. In addition, the dose of each drug which produced minimal evidence of overt neurotoxicity in 50% of animals (TD_{50}) was determined and protective indexes (TD_{50}/ED_{50}) were calculated. On the basis of the results obtained the following conclusions appear justified:

1. The TD_{50}s for the three experimental drugs are significantly higher than those for phenantoin and diphenylhydantoin.

2. The N-substituted compounds exhibit activity by all three tests; but, except for diphenylhydantoin by the s.c. Met. test and No. 2 by the l.f. EST tests, they are less potent than the two clinically employed agents. Number 1 is more effective in elevating seizure threshold than in modifying maximal seizure pattern, whereas diphenylhydantoin is more effective in modifying maximal seizure pattern than in elevating seizure threshold. Number 2, No. 3, and phenantoin exhibit no striking selectivity of action.

3. On the basis of P.I.s, No. 1 appears to be the most promising of the three experimental drugs tested. The P.I. for No. 1 by the MES test is significantly lower than that for phenantoin or diphenylhydantoin, but by the l.f. EST and s.c. Met. tests its P.I.s are significantly higher than those for diphenylhydantoin and not significantly different from those for phenantoin.

4. Mono-methyl substitution (No. 1 and phenantoin) exerts a more favorable influence on anticonvulsant activity than does either dimethyl (No. 2) or mono-ethyl (No. 3) substitution. Except for activity as measured by the l.f. EST test, the drug with methyl substitution in the 3 position (phenantoin) is more neurotoxic and exhibits significantly more anticonvulsant activity than the compound with the methyl group in position 1 (No. 1).

5. No. 1 appears worthy of more definitive clinical trial.

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