Effect of Chlorination on the Anticonvulsant Activity of Phenobarbital

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The lethal doses, neurotoxic doses, anticonvulsant potencies, and protective indices for two chlorinated barbiturates, 5-(4-chlorophenyl)-5-ethylbarbituric acid (monochloro) and 5-(3,4-dichlorophenyl)-5-ethylbarbituric acid (dichloro), were determined in mice and the results compared with similar values concomitantly determined for phenobarbital. Chlorination was found to delay markedly the time for peak anticonvulsant effect and to prolong duration of action. The lethal dose for monochloro was somewhat larger and that for dichloro significantly smaller than that for phenobarbital. The minimal neurotoxic dose for monochloro was significantly larger than that for either dichloro or phenobarbital. All three compounds exhibited anticonvulsant activity in nontoxic doses. Although chlorination of phenobarbital had no significant effect on protective indices, the protective indices for dichloro tended to be somewhat higher than those for the other two compounds.

NUMEROUS compounds have been halogenated in an effort to improve their pharmacological properties and/or margins of safety. To cite a few examples, some anti-inflammatory steroids (1), thiazide diuretics (2), antihistaminics (3), and phenothiazine ataractics (4) have been subjected to such chemical manipulation. Therefore, the availability of two chlorinated analogs of phenobarbital provided an opportunity to study the effects of chlorination on the anticonvulsant activity of an agent with well-established clinical usefulness.

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: 5-(4-chlorophenyl)-5-ethylbarbituric acid (monochloro), 5-(3,4-dichlorophenyl)-5-ethylbarbituric acid (dichloro), and 5,5-phenylethylbarbituric acid (phenobarbital). All three agents were administered orally in 0.9% sodium chloride solution; but, in order to increase the solubility of the two chlorinated compounds, two drops of a 10% solution of sodium hydroxide were added to each 50 ml. of solvent.

Two tests were utilized to determine anticonvulsant potencies (ED_{50}'s): one electrical and one chemical. The test based on electrically induced convulsions measured the ability of the drug to prevent the hindleg tonic-extensor component of maximal electroshock seizures evoked by supramaximal current (MES test; 50 ma. alternating current, 0.2-sec. stimulus duration, corneal electrodes). 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). The details of the techniques, the end points employed in mice, and the characteristics of the electroshock apparatus have been published elsewhere (5, 6). The dose lethal to 50% of animals within 24 hours after drug administration (LD_{50}) and the mean neurotoxic dose (TD_{50}) were also determined for each drug. The end point used to estimate minimal neurotoxicity was muscular incoordination, based on the inability of the animal to remain for one minute on a horizontal rod rotating at 6 r.p.m. Each drug was tested at the time of peak activity as measured by the maximal electroschock seizure (MES) test. For the determination of the ED_{50}, TD_{50}, or LD_{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, minimal neurotoxicity, or lethality, respectively. The results obtained were 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}, LD_{50}, 95% fiducial limits, and protective index (P.I. = TD_{50}/ED_{50}) were calculated by the method of Litchfield and Wilcoxon (7).

RESULTS

The time courses of anticonvulsant activity, as measured by the MES test, are shown in Fig. 1.

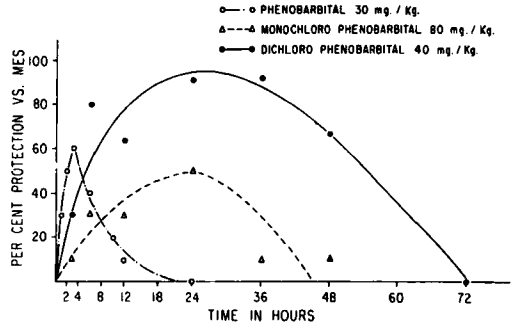

Fig. 1.—The anticonvulsant activity for phenobarbital and its monochloro and dichloro analogs.

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TABLE I.—EFFECT OF CHLORINATION ON THE NEUROTOXICITY AND ANTICONVULSANT PROPERTIES OF PHENOBARBITAL

Drug	Toxicity		Anticonvulsant Activity			
			MES Test		s.c. Met. Test	
	LD_{50}[a] mg./Kg.	TD_{50}[b] mg./Kg.	ED_{50} mg./Kg.	P.I.	ED_{50} mg./Kg.	P.I.
Phenobarbital	250 (219–285)	70 (59–83)	27.5 (22.2–34.1)	2.55 (1.92–3.35)	24.5 (19.3–31.1)	2.86 (2.12–3.86)
Monochloro	290 (259–325)	215 (169–273)	125 (106–148)	1.72 (1.27–2.32)	60 (48–75)	3.58 (2.59–4.94)
Dichloro	190 (158–220)	84 (74–96)	26.5 (17.4–40.2)	3.16 (2.06–4.88)	18.4 (12.5–27.1)	4.57 (3.04–6.84)

[a] Twenty-four-hour observation period. [b] Minimal neurological toxicity. Values in parentheses are 95% fiducial limits.

As indicated in this figure, chlorination markedly altered the time of peak anticonvulsant activity and the duration of action. Phenobarbital exerted its peak anticonvulsant effect 3 hours after oral administration, whereas the two chlorinated analogs exhibited maximum activity 24 hours after such treatment. Thus, chlorination extended the time of peak response approximately eightfold. Chlorination of phenobarbital also prolonged the duration of anticonvulsant activity. As may be calculated from the figure, it required 5 hours for the peak anticonvulsant activity of phenobarbital to fall 50%, whereas it required 14 and 30 hours for the activity of monochloro and dichloro, respectively, to decline to a similar degree.

The LD_{50}'s, TD_{50}'s, anticonvulsant ED_{50}'s, and protective indices for the chlorinated compounds, in comparison with those for phenobarbital, are shown in Table I. Except for the LD_{50}'s, which were determined 24 hours after drug administration, all tests were conducted at the time of peak drug activity as derived from the figure (3 hours for phenobarbital and 24 hours for the two chlorinated compounds). Chlorination had no dramatic effect on the LD_{50} for phenobarbital. The LD_{50} for monochloro was somewhat higher than that for phenobarbital, whereas the LD_{50} for dichloro was on the borderline of being significantly lower. Dichloro and phenobarbital induced overt symptoms of neurotoxicity in 50% of the animals after doses of 84 and 70 mg./Kg., respectively, whereas the TD_{50} for monochloro was significantly larger (215 mg./Kg.) than that for phenobarbital.

With regard to anticonvulsant potencies, the data in Table I indicate that monochloro was considerably less potent than either phenobarbital or dichloro by the two tests utilized. For example, the ED_{50} and 95% fiducial limits by the MES test were 27.5 (22.2–34.1) mg./Kg. for phenobarbital and 125 (106–148) mg./Kg. for monochloro. However, there was no significant difference between the ED_{50} for phenobarbital and that for dichloro by either the MES test or the s.c. Met. test.

When compared on the basis of protective indices (P.I.'s), it may be seen that the two chlorinated compounds do not differ significantly from phenobarbital. The P.I.'s by the MES test were 1.72 and 3.16 for monochloro and dichloro, respectively, as compared to 2.55 for phenobarbital. On the other hand, the P.I.'s as determined from data obtained by the s.c. Met. test were 2.86, 3.58, and 4.57 for phenobarbital, monochloro, and dichloro, respectively. The P.I.'s of all three compounds were higher by the s.c. Met. test than by the MES test, but the difference was significant only for monochloro.

DISCUSSION

The data presented indicate that chlorination of phenobarbital extends the time required for the drug to exert peak anticonvulsant effect, prolongs the duration of anticonvulsant action, alters the ratio of the LD_{50} to the TD_{50}, and may modify anticonvulsant activity. These observations are in agreement with the findings of Gibson and coworkers (8) in rats. The time required to reach peak anticonvulsant activity was approximately 8 times longer for monochloro and dichloro than for phenobarbital. The time required for peak anticonvulsant activity to fall 50% was found to be 3 and 7 times longer for monochloro and dichloro, respectively, than for phenobarbital.

It is interesting to note that the dose fatal to 50% of mice within a 24-hour time period is not inextricably related to the dose which causes minimal neurological deficit in a similar percentage of animals. For example, the TD_{50} for monochloro was significantly higher than that for phenobarbital, whereas the LD_{50}'s for the two compounds were not significantly different. On the other hand, the LD_{50} for dichloro was significantly lower than that for phenobarbital, whereas the TD_{50}'s for the two compounds were not significantly different. Thus, the ratio of the LD_{50} to the TD_{50} is 3.6, 1.3, and 2.3 for phenobarbital, monochloro, and dichloro, respectively. This indicates that chlorination modifies the lethal dose and the minimal neurotoxic dose independently.

With respect to anticonvulsant activity, monochloro is significantly less potent than either phenobarbital or dichloro. There is no significant difference in the anticonvulsant activity of phenobarbital and dichloro as estimated by the two tests employed.

An evaluation of the P.I.'s derived from the experimental data indicates that all the drugs have a higher margin of safety by the s.c. Met. test than by the MES test, but this difference is significant only in the case of monochloro. The P.I. for monochloro is less than that for phenobarbital by the MES test and more than that for phenobarbital by the s.c. Met. test. On the other hand, the P.I.'s for dichloro tend to be higher by both tests than those for phenobarbital, but the differences are not statistically significant.

A study of structure-activity relations suggests that 4-phenyl chlorination of phenobarbital decreases minimal neurotoxicity and anticonvulsant potency, whereas 3,4-dichlorophenyl substitution has no significant effect on either minimal neurotoxicity or anticonvulsant potency. The chlorination of phenobarbital markedly prolongs the dura-

tion of anticonvulsant activity. The more favorable anticonvulsant effects were observed when the phenyl ring of phenobarbital was chlorinated in the 3,4 positions.

Preliminary clinical trial (9) suggested that dichloro in a dose of 300 mg. per day has no significant anticonvulsant activity in man. This was unexpected since the results presented herein for mice and those reported by Gibson, *et al.* (8), for rats indicate that, except for its temporal properties, dichloro has a profile of anticonvulsant action remarkably similar to that of phenobarbital. In view of this dichotomy and because of the well-known usefulness of phenobarbital in epilepsy, the clinical value of dichloro should be unequivocally determined in order to test the validity of current laboratory procedures for screening potentially useful antiepileptic drugs.

SUMMARY

The anticonvulsant potencies (ED_{50}'s) of two experimental chlorinated barbiturates, 5-(4-chlorophenyl)-5-ethylbarbituric acid (monochloro) and 5-(3,4-dichlorophenyl)-5-ethylbarbituric acid (dichloro), and for phenobarbital were determined in mice by the following two tests: maximal electroshock seizure pattern (MES) test and pentylenetetrazol (Metrazol) seizure threshold (s.c. Met.) test. In addition, the dose of each drug fatal to 50% of animals (LD_{50}) and the dose which induced minimal evidence of neurotoxicity in 50% of animals (TD_{50}) were determined. Protective indices (P.I. = TD_{50}/ED_{50}) were calculated. On the basis of the results obtained the following conclusions appear to be justified.

1. Chlorination of phenobarbital modifies the lethal dose independently of the minimal neurotoxic dose.

2. Chlorination extends approximately eightfold the time required for the drug to exhibit peak anticonvulsant activity and prolongs three- to sevenfold the time for anticonvulsant activity to fall 50%.

3. The chlorinated barbiturates exhibit anticonvulsant activity by both tests; *monochloro* is less potent than phenobarbital, whereas the anticonvulsant potency of dichloro is not significantly different from that of phenobarbital.

4. On the basis of P.I.'s, dichloro exhibits the most favorable indices, but the P.I.'s for dichloro are not significantly different from those for phenobarbital. The P.I.'s for all three compounds are higher by the s.c. Met. test than by the MES test, but this difference is significant only in the case of monochloro.

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Structural Studies on the Triterpene Obliquol

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Oxidation studies have indicated that the two hydroxyl groups of obliquol are secondary, one of which exhibits some hindrance as in the Oppenauer oxidation. Nuclear magnetic resonance studies have supported the chemical evidence that the second hydroxyl group is in the 12 position in obliquol. The C-18 methyl peak of obliquol diacetate has shifted downfield from the corresponding peak in lanosterol acetate due to perturbation by the 12-acetyl group. Therefore, it was concluded that obliquol has the structure I. The complete NMR spectra of obliquol, lanosterol, and their acetates are interpreted.

IN A PREVIOUS communication (1) there was reported the isolation of a new triterpene, obliquol (I), from the fungus, *Poria obliqua* (Bres.). At that time, obliquol (I) was shown to be a tetracyclic triterpene with two hydroxyl groups. It was shown to possess two double bonds, one of which was shown to be unreactive and assigned to the 8,9 position. The possibility, based on infrared data, was proposed that one of the hydroxyl groups was primary. This has since been found to be a secondary hydroxyl group. Additional studies on the structure of obliquol comprise the contents of this communication.

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