Comparison of Anticonvulsant Potencies of Cyheptamide, Carbamazepine, and Phenytoin

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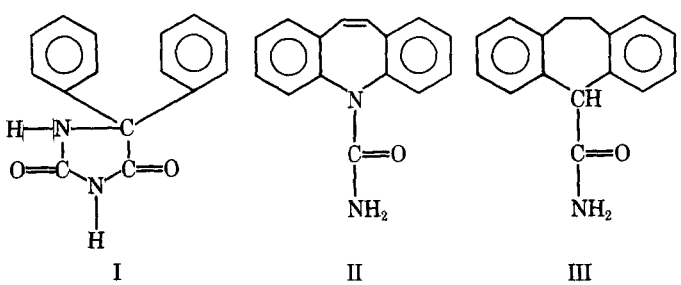
Abstract □ Carbamazepine and cyheptamide have certain stereochemical features in common with phenytoin; when superimposed, two bulky hydrophobic groups in each permit the approximate apposition of two electron donor atoms. The anticonvulsant activity of each compound was determined in mice using a standard maximal electroshock procedure, and the relative potencies are expressed in terms of the blood and brain concentrations as well as the intraperitoneal dosage. Although cyheptamide was much less potent than carbamazepine or phenytoin on the basis of intraperitoneal dosage, the difference in potency was much less when blood or brain concentrations were compared. These data should be of value for quantitative structure–activity relationship studies.

Keyphrases □ Carbamazepine—anticonvulsant activity, intraperitoneal dosage, blood and brain concentrations □ Cyheptamide—anticonvulsant activity, intraperitoneal dosage, blood and brain concentrations □ Phenytoin—anticonvulsant activity, intraperitoneal dosage, blood and brain concentrations □ Anticonvulsant activity—carbamazepine, cyheptamide, phenytoin, blood and brain concentrations □ Structure–activity relationships—carbamazepine, cyheptamide, and phenytoin compared for anticonvulsant activity

A stereochemical basis of anticonvulsant drug action was described (1–5) for various chemically unrelated molecules. When brought into apposition, it becomes apparent that molecules as chemically unrelated as phenytoin (I) and diazepam not only have bulky hydrophobic groups with similar orientations in space but also have electron-donating groups in similar spatial positions. By using space-filling and Dreiding stereomodels, it can be seen that carbamazepine (II) and cyheptamide (III) (10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-5-carboxamide) also bear obvious structural similarities to phenytoin. The two phenyl rings in each, when approximately superimposed, permit the reasonable apposition of a carbonyl oxygen as well as a second electron donor, the imide nitrogen in phenytoin and the amide nitrogen in carbamazepine and cyheptamide.

Although several reports (6–8) have dealt with the pharmacological properties of cyheptamide, the procedures employed do not permit direct comparison of its anticonvulsant activity with that of carbamazepine, phenytoin, and other structurally related anticonvulsants. The present investigation presents such data for comparative purposes. In a preliminary experiment, the ED_{50} of cyheptamide after intraperitoneal administration was 81.0 mg/kg (based on a maximal electroshock technique). According to Krall *et al.* (9), this value would represent a potential antiepileptic worthy of further study. Therefore, the relative potencies of cyheptamide, carbamazepine, and phenytoin were determined with respect to blood and brain concentrations in addition to intraperitoneal dosage. The results should be of value in quantitative structure–activity analysis involving the particular class of compounds known to conform to the previously mentioned stereochemistry.



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EXPERIMENTAL

Male CF1 mice[1], 30–32 days old when received, were allowed to acclimate for several days after possible food and water deprivation during transportation. They were permitted food and water *ad libitum* except during the short time when removed from their cages for testing.

Carbamazepine[2] and phenytoin sodium[3] were suspended in 30% aqueous polyethylene glycol 400, and cyheptamide[4] was suspended in 5% gum acacia. The suspensions were sonicated for ~5 min to produce a fine suspension. The drugs were administered intraperitoneally (0.01 ml/g) to mice weighing an average of 22 g.

Each compound was assayed at the time of its peak anticonvulsant effect. A maximal electroshock (MES) test (10) was employed for the peak time determinations as well as for subsequent dose–response experiments. Briefly, this test is a measure of the ability of a drug to abolish the hindlimb extensor component of the seizure pattern induced by a 60-Hz alternating current of 50 mamp delivered for 0.2 sec *via* corneal electrodes. The animal was restrained only by hand and was released at the moment of stimulation to permit observation of the seizure throughout its entire course. Protection against the extensor component beyond a 90° angle with the trunk was required for an anticonvulsant effect to be registered. An approximate ED_{50} of the drug to be tested was given to groups of five mice, and the maximal electroshock test was performed 15 min after administration and at 30-min intervals thereafter until the time of peak effect had obviously passed (indicated by fewer animals being protected than at an earlier time). All subsequent dose–response determinations were based on assays of the drug at its established peak time.

Immediately after the maximal electroshock test, 100 μl of blood was collected in 2% sodium fluoride and the animal was decapitated. The entire brain was removed and blotted on filter paper, and the specimens (including blood) were stored at −80° until they were assayed. Blood and brains from 10 mice were pooled for each dose, and at least five doses were employed for each drug.

The blood and brain concentrations for each phenytoin dose were assayed by GLC with 5-(*p*-methylphenyl)-5-phenylhydantoin as an internal standard. The blood and homogenized whole brain specimens were saturated with ammonium sulfate, acidified, and extracted with toluene. The toluene layer was separated and back-extracted with tetramethylammonium hydroxide according to the procedure of Johnson *et al.* (11). The samples were analyzed on 3% OV-17 on 80–100-mesh Supelcoport at 230° and detected by flame ionization (injector port and detector temperature 300°).

Carbamazepine was extracted from alkalinized blood and homogenized whole brain specimens by *n*-butyl chloride. The *n*-butyl chloride was

[1] From Charles River Breeding Laboratories.
[2] Geigy Pharmaceuticals.
[3] Warner-Lambert Co.
[4] Pierce Chemical Co.

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Table I—Anti-Maximal Electroshock Potencies of Phenytoin, Carbamazepine, and Cyheptamide

ED_{50}[a]	Phenytoin	Carbamazepine	Cyheptamide
Intraperitoneal, mg/kg	7.1 (6.5–7.8)	9.7 (8.5–11.0)	81.0 (52.0–127.0)
Blood, μg/ml	2.2 (1.8–2.8)	2.4 (2.2–2.7)	5.5 (3.9–7.8)
Brain, μg/g	5.3 (4.7–5.9)	4.9 (4.1–5.7)	8.9 (7.5–10.6)

[a] The ED_{50} values are listed with 95% confidence intervals in parenthesis. Potencies are expressed in terms of the intraperitoneal dose and blood and brain concentrations.

evaporated to dryness, and the residue was derivatized with pentafluorobenzoyl chloride to increase sensitivity by electron capture. 10-Methoxycarbamazepine was added as an internal standard. The samples were analyzed by the method described by Schwertner *et al.* (12).

Cyheptamide was extracted from blood and brain specimens in the manner described for carbamazepine. The *n*-butyl chloride was evaporated to dryness and reconstituted in 50 μl of chloroform. Carbamazepine was used as an internal standard. The analysis was performed on 3% OV-17 on 80–100-mesh Supelcoport at 250° and detected by flame ionization (injector port temperature 270° and detector temperature 300°). A typical chromatogram for cyheptamide extracted from brain tissue is depicted in Fig. 1.

The anticonvulsant data were evaluated with 95% confidence limits by the statistical method of Litchfield and Wilcoxon (13). The relative anticonvulsant potencies (ED_{50}) are expressed in terms of the blood and brain concentrations as well as the intraperitoneal dosage.

RESULTS AND DISCUSSION

The comparative times of peak anticonvulsant effect of carbamazepine, cyheptamide, and phenytoin were ~30, 45, and 60 min, respectively. Although the anti-maximal electroshock potency of carbamazepine was similar to that of phenytoin, the potency of cyheptamide was considerably less (Table I and Fig. 2). The dose of cyheptamide necessary to protect 50% of the mice from tonic hindlimb extension (ED_{50}) was 81.0 mg/kg (95% confidence limits of 52.0–127.0), expressed as the intraperitoneal dose. The respective ED_{50} values (95% confidence limits) for carbamazepine and phenytoin were 9.7 (8.5–11.0) and 7.1 (6.5–7.8) mg/kg, respectively (Table I). However, when the blood and brain concentrations of the three drugs were compared, the relative potencies were not that

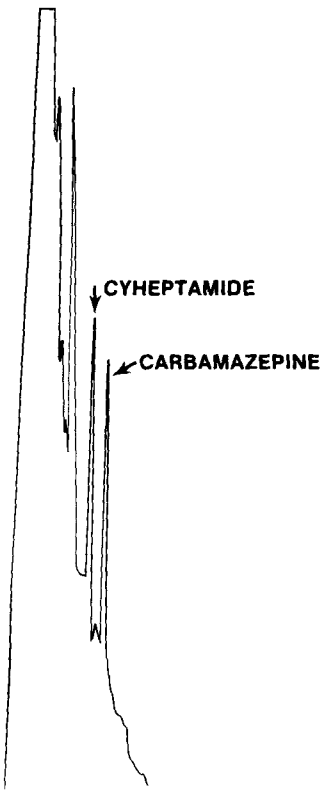

Figure 1—*Gas–liquid chromatogram of cyheptamide from brain extract. The internal standard was carbamazepine.*

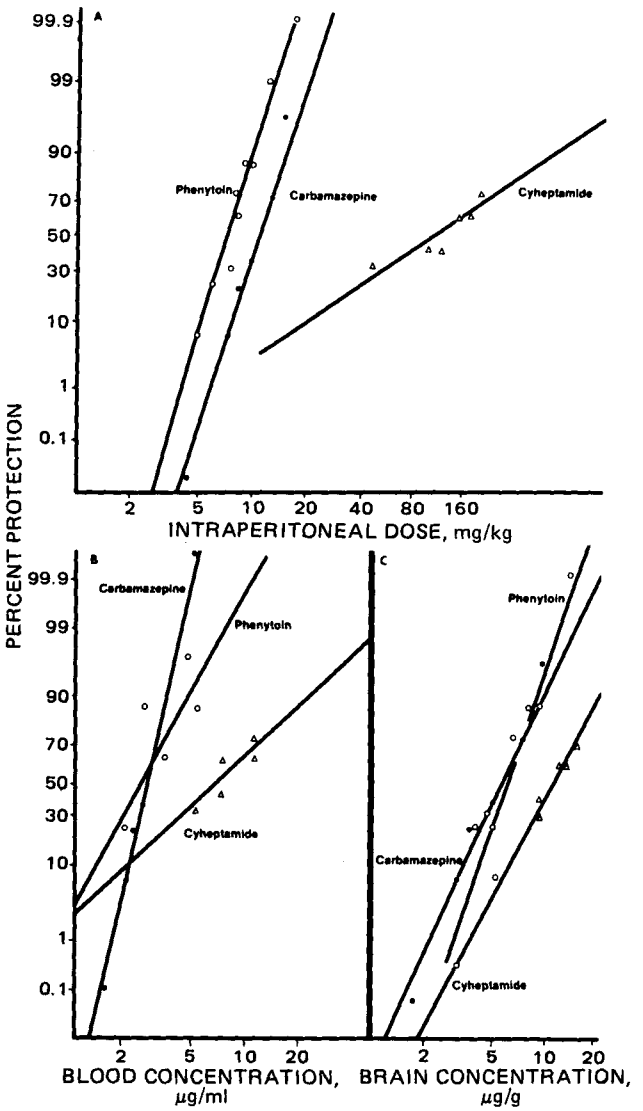

Figure 2—*Log dose–response curves for phenytoin, carbamazepine, and cyheptamide expressed in terms of the intraperitoneal dosage (A), blood concentration (B), and brain concentration (C).*

dissimilar. Cyheptamide again was the least potent (Table I). But the potency ratio (95% confidence limits) of cyheptamide to phenytoin, 11.4 (7.3–17.9) on a milligram per kilogram basis, was only 2.5 (1.6–3.8) when blood concentrations (micrograms per milliliter) were compared. When brain concentrations (micrograms per gram) were compared, the ratio was even less, 1.7 (1.4–2.1). In the comparison of cyheptamide to carbamazepine, the ratios were 8.3 (6.1–11.6) on a milligram per kilogram basis, 2.3 (1.7–2.9) for blood concentrations, and 1.8 (1.8–1.9) for brain concentrations. Phenytoin was only slightly more potent than carbamazepine on a milligram per kilogram basis ($p = 0.05$), but the difference was not significant based on blood or brain concentrations.

The large difference in the ED_{50} (intraperitoneal) for cyheptamide might be explained by its lipophilicity. Calculations of log *P* based upon hydrophobicity constants (14) produce an estimated partition coefficient of 550[5] (log $P = 2.74$). The compound is so lipid soluble that it might not achieve the same aqueous dispersion as phenytoin or carbamazepine when given intraperitoneally; thus, absorption would be relatively less due to the smaller effective surface area to which the drug is exposed. The calculated partition coefficients[5] for phenytoin and carbamazepine were fairly similar, 170 (log $P = 2.23$) and 151 (log $P = 2.18$), respectively. This

[5] Log *P* calculations: cyheptamide, from log P $(C_6H_5)_2CH_2 + \frac{1}{3}\pi$(cyclohexane) $+ \pi(CONH_2) = 4.14 + 0.84 + (-2.24) = 2.74$; carbamazepine, from log P $(C_6H_5)_2NCONH_2 + \frac{1}{3}\pi(C_6H_5) = 1.53 + 0.65 = 2.18$; phenytoin, from log P (hydantoin) $+ 2\pi(C_6H_5) = -1.69 + 3.92 = 2.23$. Note that the calculated log P for phenytoin is slightly different than the experimental value of 2.47 (14). Only calculated values were used to maintain consistency.

result may explain their similar potencies when their ED_{50} values are compared by any of the three criteria.

An alternative explanation of the potency difference, also based on relative lipophilicities, might be the parabolic dependence of potency on hydrophobicity described by Penniston *et al.* (15) and McFarland (16). Although potency among various compounds is known to increase with increasing lipophilicity, there is frequently an optimum hydrophobicity beyond which potency actually decreases. A quantitative description of this phenomenon is expressed according to (17):

$$\log \frac{1}{C} = -k(\log P)^2 + k' \log P + k'' \qquad \text{(Eq. 1)}$$

Although various explanations might account for such behavior, the following concept is popular. A certain degree of lipophilicity is necessary so that molecules may "dissolve" in and penetrate the lipid matrix of membranes. However, extremely lipophilic substances may actually take up "residence" within the lipid matrix of the membrane. Each membrane interposed along the path a drug might follow to its receptor thus lessens the probability that the drug will reach the receptor. The greatest reduction in such probability occurs at extreme values of the partition coefficient. Drugs with low partition coefficients may have difficulty entering the membrane, while those with high coefficients may not leave readily. Thus, the rather large increase in the partition coefficient of cyheptamide relative to phenytoin and carbamazepine might be responsible for its significantly lower potency. Furthermore, it might be assumed that fewer membranes are interposed between the bulk drug concentrations and the receptors when drug activity is expressed in terms of brain concentrations than when expressed as the intraperitoneal dose or blood concentration. Therefore, the smaller potency ratios observed for the brain concentrations are likely an expression of the smaller difference in probability permitted by fewer barriers separating the drug from its receptor.

The higher brain to blood concentration ratio for phenytoin (2.4) in comparison with carbamazepine (2.0) might reflect its slightly more lipophilic character, but a greater binding of carbamazepine to plasma protein also might be involved. Because cyheptamide is the most lipophilic of these compounds, its surprisingly low brain to blood ratio (1.6) is probably the result of substantial binding to plasma protein.

Previous investigations of cyheptamide produced significantly lower potency estimates than the present data indicate. The probable explanation for this difference points to the problem many investigators have when correlating anticonvulsant structure with activity, namely, the failure to apply a uniform experimental model of epilepsy. In the earliest study (6), mice were subjected to a maximal electroshock procedure in which 30 mamp of 60-Hz current was applied for 0.2 sec *via* corneal electrodes. The ED_{50} value was 25 ± 1 mg/kg by the intraperitoneal route and 33 ± 3.5 mg/kg by the oral route. A later report cited an ED_{50} (oral) of 38 mg/kg (7). In that study, the current was only 25 mamp (60 Hz), again delivered for 0.2 sec through corneal electrodes. The lower potency of cyheptamide determined in the present study is likely the result of the higher (50 mamp) current, as prescribed in the most thoroughly documented model of grand mal epilepsy (9, 18).

In summary, the relatively low anti-maximal electroshock potency described previously (6, 7) for cyheptamide has been verified. However, its potency is not so discrepant from that of structurally related carbamazepine and phenytoin as might be inferred from the intraperitoneal dosage. The ED_{50} values expressed in terms of tissue concentrations might be of greater value in a quantitative structure–activity investigation than those values based on parenteral dosage.

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