PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 156, 491-495 (1977)

The Distribution between Plasma and Brain of Glutethimide and 4-Hydroxyglutethimide following Their Administration to Mice (39964)

K. A. KENNEDY, A. R. HANSEN, AND L. J. FISCHER[1,2]

The Toxicology Center, Department of Pharmacology, The University of Iowa, Iowa City, Iowa 52242[1]

Introduction. Previous reports (1) have shown that a large fraction of glutethimide was metabolized by humans to 4-hydroxyglutethimide (4-hydroxy-2-ethyl-2-phenylglutarimide). This metabolite accumulated in the plasma of glutethimide-intoxicated patients and was found in high concentrations in the brains of some cases of fatal glutethimide poisoning (2, 3). Pharmacological studies in mice showed that 4-hydroxyglutethimide isolated from the urine of dogs given glutethimide possessed twice the sedative hypnotic activity of glutethimide (as measured by the rotarod test) and was approximately twice as toxic as glutethimide when the LD_{50}s for each compound were determined (3, 4).

The reasons why the 4-hydroxyglutethimide isolated from dog urine is more potent than glutethimide as a sedative hypnotic in mice are not known. The hydroxy metabolite may reach higher concentrations in the brain or it may possess a higher intrinsic activity. The present study was conducted to determine if there were possible differences in the distribution and elimination of glutethimide and 4-hydroxyglutethimide that could explain the higher potency of the metabolite. Plasma and brain levels were measured after sedative doses of glutethimide and 4-hydroxglutethimide and these were correlated with the plasma protein binding and partition coefficients of each compound. In addition, experiments were conducted to examine the relationships between dose, drug levels, and pharmacological effects of the two compounds. The results of these experiments help to establish the basis for the higher potency of 4-hydroxyglutethimide when compared to the parent drug.

Materials and methods. Drugs. Glutethimide powder (d,l-glutethimide), obtained from U.S. Vitamin Inc., Tuckahoe, N.Y., was used as the racemic mixture. The metabolite, 4-hydroxyglutethimide, was isolated from urine of dogs given intoxicating doses of glutethimide as described previously (4). The identity of the biosynthetically prepared 4-hydroxyglutethimide was confirmed by gas chromatography/mass spectrometry (4). Since 4-hydroxyglutethimide contains two assymetric carbon atoms, it may exist as a mixture of isomers. The isolated material behaved as a pure substance upon chromatographic analysis (4) and thus represented a diastereoisomer (i.e., mixture of two optical isomers) or a single optical isomer. Keberle *et al.* have concluded that 4-hydroxyglutethimide produced in the dog arises from the d-isomer of racemic glutethimide (5). This means that the material used in the present study was a single optical isomer.

Radiolabeled compounds were used for the octanol/water partition coefficient experiments and the protein binding experiments. Radiolabeled [^{14}C]glutethimide (at the C-1 carbon of the glutarimide ring) was obtained from New England Nuclear Inc., Boston, Mass., with a specific activity of 1.61 mCi/mmol. Radiolabeled 4-[2-^{14}C]-hydroxyglutethimide was obtained from the urine of dogs given [^{14}C]glutethimide (4). The specific activity of 4-[2-^{14}C]hydroxyglutethimide was 0.009 mCi/mmole. Purity of all administered drugs and radiochemicals was determined to be greater than 95% by thin-layer chromatography on silica gel GF plates, 250 μm (Analabs) in three solvent systems: hexane:ether:acetic acid (70:30:8), chloroform:acetone (90:10), and dichloromethane:dioxane (90:10). Thin-layer chromatograms of radiolabeled glutethimide in each of the solvent systems were scanned by a Packard radiochromatogram scanner. For either drug,

[1] H. E. Williamson, Sponsor.

[2] Send reprint requests to Dr. Lawrence J. Fischer, The Toxicology Center, Department of Pharmacology, The University of Iowa, Iowa City, Iowa 52242.

ISSN 0037-9727

a single radioactive peak was observed and was coincident with authentic glutethimide or 4-hydroxyglutethimide.

Partition coefficients. The relative lipophilicity of glutethimide and 4-hydroxyglutethimide was determined by partitioning 100 μg of the ^{14}C-labeled drugs between octanol and 0.1 *N* phosphate buffer, pH 7.4, at room temperature. The buffer and octanol were equilibrated with each other overnight before use. Each drug was added to separate tubes containing 2 ml of the phosphate buffer. Two milliliters of octanol was added and the samples were shaken at room temperature for 4 hr. After partitioning, the organic and aqueous phases were separated and counted in a Packard Model 3400 liquid scintillation counter, using a dioxane-based scintillant containing 100 g of naphthalene, 7 g of 2,4-diphenyloxazole, and 0.3 g of 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene per liter of solvent. Counting efficiency was determined using the internal standard method.

Animals. Male Swiss Webster mice (20–25 g) were used in the plasma protein binding experiments and in the tissue distribution experiments. Animals were housed in stainless-steel cages in a controlled environment and fed Purina Lab Chow blocs and water *ad libitum*.

Plasma protein binding. Pooled mouse plasma was used to study the binding of glutethimide and 4-hydroxyglutethimide to proteins using the equilibrium dialysis method described by Curry *et al.* (6). One milliliter of mouse plasma containing either 4-hydroxyglutethimide or glutethimide was dialyzed against 4 ml of buffer. Equilibrium dialysis was carried out at concentrations of 0.04 and 0.14 μmole of glutethimide or 4-hydroxyglutethimide/ml of plasma. The lower concentration was chosen to represent the peak plasma level of either drug observed in the pharmacokinetic studies and the higher concentration of the two drugs was used to determine if plasma protein binding was saturated at these levels. Preliminary experiments showed that equilibrium was achieved after 18 hr of dialysis.

ED_{50} experiments. The potency of 4-hydroxyglutethimide and glutethimide was compared using the rotarod method as previously described (4). Mice were trained to maintain themselves on a slowly rotating wooden rod for at least 5 min. On the next day, groups of mice were given either glutethimide or 4-hydroxyglutethimide dissolved in dimethylacetamide by intraperitoneal injection (0.001 ml/g body weight). Dose intervals were selected on a logarithmic scale and encompassed the known ED_{50} (4). At the time of peak effect (13–16 min) the mice were placed on the rotating wooden rod, and an animal that fell twice in a 2-min period was considered to be ataxic. Immediately at the end of the test period (13–16 min after dosing) the animals were sacrificed by decapitation. Blood and brain tissue were collected and immediately frozen on dry ice for subsequent assay.

Pharmacokinetic studies. Mice were administered intraperitoneally 215 μmole/kg doses of glutethimide or of 4-hydroxyglutethimide dissolved in corn oil. Animals were lightly anesthetized with ether, and blood was obtained at the time of sacrifice by cardiac puncture at 10, 30, 60, 180, and 360 min after administration of the drugs. Whole blood was separated into plasma and cells by centrifugation. The brains were homogenized in 7 vol of distilled water. All samples were kept frozen until assayed for glutethimide or 4-hydroxyglutethimide. The concentrations of drugs in plasma blood and brain were determined by the gas chromatographic method of Hansen and Fischer (7).

Results. The partition coefficients (octanol/water) for glutethimide and 4-hydroxyglutethimide are shown in Table I. The

TABLE I. PLASMA PROTEIN BINDING AND OCTANOL/WATER PARTITION COEFFICIENTS FOR GLUTETHIMIDE AND 4-HYDROXYGLUTETHIMIDE.

Drug	Octanol/water partition coefficient[a]	Plasma protein binding (% bound)[a]	
		0.04 μmole/ml	0.14 μmole/ml
Glutethimide	46	63	74
4-Hydroxyglutethimide	17	43	50

[a] Data shown are means of duplicate trials. Variation between trials was less than 10% of the mean of each duplicate.

data indicate that in this system, glutethimide possesses a partition coefficient 2.5 times that of 4-hydroxyglutethimide. The data from the protein binding experiments are also shown in Table I. The extent of drug binding to plasma proteins was similar at the two concentrations studied, indicating that the binding sites on plasma proteins were not saturated at concentrations attained in these studies. The plasma protein binding experiments, while showing that glutethimide was somewhat more extensively bound than was 4-hydroxyglutethimide, clearly indicate that a large fraction of either drug was present in the unbound state and readily available for equilibration with other tissues.

Concentrations of glutethimide and 4-hydroxyglutethimide in brain and plasma following a 215 μmole/kg dose of each drug separately are shown in Fig. 1. The peak concentrations of both 4-hydroxyglutethimide and glutethimide in brain or plasma occurred between 10 and 30 min after drug administration. This time interval correlates with the period of drug-induced loss of righting reflex observed in the 4-hydroxyglutethimide-treated mice. Similarly, the mice given glutethimide became ataxic between 10 and 30 min after drug administration, although these mice did not lose the righting reflex.

Both glutethimide and 4-hydroxyglutethimide declined at similar rates from the plasma and brain of mice, as shown in Fig 1. The half-life for the disappearance of either drug from the plasma was about 65 min. The half-life for disappearance of glutethimide or 4-hydroxyglutethimide from the brain was approximately 40–45 min. No glutethimide or 4-hydroxyglutethimide was detected in the brains of animals sacrificed 360 min after drug administration. In mice administered glutethimide, only small amounts (0.009 μmole/ml) of 4-hydroxyglutethimide were detected in plasma. No

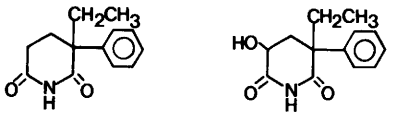

FIG. 1. The structures of glutethimide and 4-hydroxyglutethimide.

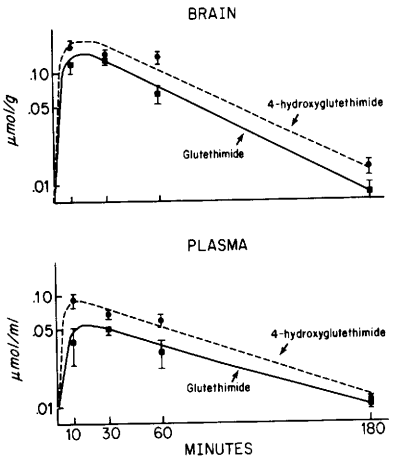

FIG. 2. The elimination of glutethimide (■) and 4-hydroxyglutethimide (●) from the brain and plasma of mice given 215 μmole/kg, ip. Each point represents the mean drug concentration in brain or plasma for three animals. Means of plasma and brain concentrations for glutethimide were compared to 4-hydroxyglutethimide concentrations at each time point by Student's *t* test (8). No significant differences were obtained.

4-hydroxyglutethimide was detected in the brains of animals which received glutethimide.

Table II demonstrates the relationships among the administered dose, the degree of biological effect produced, and the concentration of drug attained in both brain and whole blood following treatment of mice with either glutethimide or 4-hydroxyglutethimide. These data show that with either glutethimide or 4-hydroxyglutethimide, as the dose in increased, a greater sedative-hypnotic effect is produced, along with a corresponding increase in the concentration of active drug in brain and blood. The ratios of the concentrations of either glutethimide or 4-hydroxyglutethimide between blood and brain were similar and remained constant with increasing doses. The concentration of 4-hydroxyglutethimide in brain or blood following the ED_{50} dose is approximately half the concentration of glutethimide, given at its respective ED_{50} dose.

Discussion. The data presented here demonstrate that for either glutethimide or 4-hydroxyglutethimide there is a good correlation between drug effect and drug concen-

TABLE II. Relationship among Dose, Biological Activity, and Drug Concentration in the Brains and Blood of Mice Given Glutethimide or 4-Hydroxyglutethimide.

Dose[a]	No. of ataxic animals	Brain[b]	Blood[b]	Ratio (brain/blood)
		Glutethimide		
144.2	0 (6)[c]	46 ± 1[d]	29 ± 2	1.60
217	3 (6)	185 ± 8	127 ± 6	1.47
325	6 (6)	274 ± 7	157 ± 7	1.75
		4-Hydroxyglutethimide		
69	0 (5)	58 ± 2	34 ± 3	1.69
103	2 (6)	94 ± 2	68 ± 2	1.39
155	5 (6)	131 ± 4	91 ± 3	1.46

[a] Micromoles per kilogram given intraperitoneally.
[b] Nanomoles per gram of tissue or per milliliter of blood at the time of peak pharmacologic effect (13–16 min).
[c] Numbers in parentheses represent the total number of animals given drug at the indicated dose.
[d] Data shown are means ± standard errors.

tration, whether the latter is expressed as a dose level or as a concentration in brain or blood.

The hydroxymetabolite, which is more polar than glutethimide, is bound to a lesser extent to plasma proteins. Although a larger fraction of 4-hydroxyglutethimide is free to partition with brain tissue, the relatively greater lipid solubility of glutethimide may increase the uptake by brain for, empirically, each distributes between blood and brain tissue in a similar manner. The penetration of 4-hydroxyglutethimide into mouse brain is consistent with previous results showing large amounts of the metabolite in postmortem blood and brain tissue of humans who have died of glutethimide poisoning (7). The curves demonstrating the rate of disappearance of 4-hydroxyglutethimide and glutethimide from plasma and brain were similar after equimolar doses of each compound. Although the concentrations of 4-hydroxyglutethimide appeared to be slightly greater than the levels of glutethimide in both brain and blood at corresponding time points, these differences were not statistically significant. Thus, there seems to be little difference in distribution and elimination between glutethimide and its active metabolite.

The results support the assumption that a known concentration of glutethimide or 4-hydroxyglutethimide in either brain or blood can be correlated with a given degree of central nervous system depression. The data also show that the twofold-higher potency noted for 4-hydroxyglutethimide relative to glutethimide which is documented here and elsewhere (3, 4) cannot be attributed to large differences in distribution or elimination of the two compounds. Rather, it is probably a consequence of differences in the intrinsic activity of the two compounds.

Stereochemical considerations compel us to state that the above comments on the potency and elimination of 4-hydroxyglutethimide, as compared to glutethimide, pertain only to the metabolite isolated from the dog. Identical results would be obtained with 4-hydroxyglutethimide obtained from human urine if man, like the dog (5), produced this metabolite only from the d-isomer of racemic glutethimide. Keberle *et al.* (5) have suggested that this is the case, since man and dog apparently metabolize racemic glutethimide exactly in the same manner. We wish to be cautious, however, since the metabolism of the separated optical isomers of glutethimide has not been studied in man.

Summary. Following administration of d,l-glutethimide or its active metabolite, d-4-hydroxyglutethimide, the presumed d-glutethimide metabolite, to mice, the relative partition between blood and brain as well as the rate of disappearance of each substance from these tissues was found to be similar. In addition, the degree of CNS depression produced by increasing doses of either substance was directly proportional to the blood or brain level attained, indicating that the twofold-greater potency of the active metabolite, relative to glutethimide, is mostly a function of its intrinsic pharmacologic activity and not dependent upon differences in drug disposition.

1. Bütikofer, E., Cottier, P., Imhof, P., Keberle, H., Reiss, W., and Schmid, K., Arch. Exp. Pathol. Pharmakol. **244,** 97–108 (1962).
2. Ambre, J., and Fischer, L. J., Res. Commun. Chem. Pathol. Pharmacol. **4,** 307–326 (1972).
3. Hansen, A. R., Kennedy, K. A., Ambre, J. J., and Fischer, L. J., N. Engl. J. Med. **292,** 250–252 (1975).
4. Ambre, J. J., and Fischer, L. J., Drug Metab.

Disp. **2.** 151–158 (1974).
5. Keberle, H., Reiss, W., and Hoffmann, K., Experientia **18,** 105–111 (1962).
6. Curry, S. H., Bordon, J. S., Riddal, D., Gorden, J., Simpson, P., Benno, T., Rondel, R., and McMartin, B., Clin. Pharmacol. Ther. **12,** 849–851 (1971).
7. Hansen, A. R., and Fischer, L. J., Clin. Chem. **20,** 236–242 (1974).
8. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics." McGraw-Hill, New York (1960).

Received March 1, 1977. P.S.E.B.M. 1977, Vol. 156.