Physicochemical and Hydrolytic Characteristics of Phenytoin Derivatives

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To further clarify the pharmacokinetic characteristics of phenytoin (DPH) and its derivatives, DPH-1-methylnicotinate (MNDPH), valeroyl DPH (VADPH) and valproyl DPH (VPDPH), in plasma and brain, we have investigated their physicochemical properties and protein binding characteristics. Additionally, the hydrolytic conversion of these derivatives to DPH was also studied using small intestine, liver and brain tissues, as well as rat plasma. The log partition coefficient (*PC*) values of all derivatives were much higher than that of DPH. Judging from their pK_a values (5.68 and 5.91 for VADPH and VPDPH, respectively) and pH-solubilities, VADPH and VPDPH were acidic compounds, while MNDPH was basic. These data indicated that most fractions of VADPH and VPDPH existed as an ionized form (these fractions existed in an ionized form, 0.98 and 0.97, respectively) at physiological pH, whereas MNDPH existed as a unionized form under the same conditions. Rosenthal or Scatchard plots of the binding data of DPH and its derivatives to both rat plasma protein and bovine serum albumin (BSA) exhibited straight lines over their concentration ranges used, indicating that DPH and its derivatives have a single binding site on the protein. The binding potencies (*K* or $n \cdot P_i$ value) of the derivatives to both proteins were much greater than that of DPH. No DPH produced from VADPH and VPDPH was found in the biological fluids over a period of 24 h. However, the hydrolysis of MNDPH to DPH was observed in plasma and the tissues used, with the most rapid hydrolysis in the small intestine, and the hydrolysis rate constant in plasma was *ca.* 20-fold greater than that in the brain. The present results lead us to propose that the low uptake of VADPH and VPDPH into the brain, as well as their rapid elimination from plasma is mainly ascribed to both the high protein binding and the large dissociation of derivatives in the plasma, compared with that of DPH.

Keywords phenytoin; valeroyl DPH; valproyl DPH; protein binding; hydrolysis; physicochemical property

To gain access to the site of action of anticonvulsants in the brain, drugs must cross one or more barriers. In contrast to the capillary endothelial junction in other tissues, the endothelial cells in brain capillaries are joined by continuous tight intercellular junctions, indicating that drugs must pass through the cells, rather than between them, in order to be distributed into the brain.

It is generally accepted that plasma protein-bound drugs cannot cross the capillary membrane and only an unbound drug can diffuse freely out of the vascular compartment to reach the tissue compartment. It seems that an anticonvulsant drug such as phenytoin (DPH) would be restricted from crossing the blood-brain barrier (BBB) since it binds strongly to plasma proteins.¹⁾ However, the most predominant "free-drug hypothesis" in pharmacology and pharmacokinetics has been challenged by some recent studies. Pardridge *et al.* have shown that plasma protein-bound drugs could be partly available for transport into the brain.²⁾ In addition, Lin and Lin have demonstrated that plasma protein binding of benzodiazepines significantly decreased the uptake by the brain, but to a lesser extent than that predicted from the unbound drug fraction *in vitro*.³⁾ Physicochemical properties such as molecular dipole moment and partition coefficient have

also been associated with anticonvulsant activity because of their effects on the ability of a drug to pass through the BBB.⁴⁾

Recently, we have reported the pharmacokinetics of phenytoin and its derivatives in rat plasma and brain, and have shown that the distribution of the derivatives into the brain was much less than that of DPH, probably due to the high protein binding capacities, in addition to the rapid elimination of derivatives from the plasma and brain.⁵⁾ However, detailed studies of the interactions of DPH and its derivatives with albumin have not been carried out. In order to identify the major factors involved in the distribution and elimination, the physicochemical properties and protein binding of the derivatives, valproyl DPH (VPDPH) and valeroyl DPH (VADPH), were determined along with those of a newly synthesized derivative, DPH-1-methylnicotinate (MNDPH). These properties determined were discussed in relation to the pharmacokinetic and pharmacodynamic characteristics of DPH and its derivatives (Fig. 1).

MATERIALS AND METHODS

Materials Phenytoin (JP grade), valproic acid and

Fig. 1. Chemical Structures of DPH and Its Derivatives

nicotinic anhydride of reagent grade were obtained from Nacalai Tesque, Inc. (Kyoto, Japan), Tokyo Kasei Kogyo Co. (Tokyo, Japan) and Aldrich Chemical Co., Inc. (Milwaukee, WI, U.S.A.), respectively. Valeric acid and bovine serum albumin (BSA, Fraction V, fatty acid-free) were purchased from Sigma Chemical Co. (St. Louis, MO., U.S.A.). Acemethacin and medazepam, internal standards for high performance liquid chromatography (HPLC), were generously supplied by Kowa Co. (Nagoya, Japan) and Shionogi Pharmaceutical Co. (Osaka, Japan), respectively. Clonazepam and indomethacin were generous gifts of Sumitomo Pharmaceutical Co. (Osaka, Japan). Nicotinamide adenine dinucleotide phosphate (NADP) and glucose-6-phosphate (G-6-P) were obtained from Oriental Yeast Co. (Tokyo, Japan). Nicotinamide was purchased from Kishida Chemical Co., Ltd. (Osaka, Japan). All other chemicals used were of reagent or HPLC grade. Male Wistar rats weighing 200–250 g were used throughout these experiments.

Synthesis of Derivatives 3-(2-Propylpentanoyl)-5,5-diphenylhydantoin (VPDPH) and 3-pentanoyl-5,5-diphenylhydantoin (VADPH) were synthesized by the method described in our previous paper.⁵⁾

MNDPH was synthesized according to the method reported by Pop *et al.*⁶⁾ (2,4-Dioxo-5,5-diphenyl-3-imidazolidinyl)methyl pyridine-3-carboxylate (DPH-1-methyl nicotinate): colorless prisms (from acetonitrile), mp 180–181 °C. ¹H-NMR (DMSO-*d*₆): δ 5.80 (2H, s), 7.35–7.46 (10H, m), 7.55 (1H, ddd, *J*=8.0, 5.0, 1.0 Hz), 8.21 (1H, ddd, *J*=8.0, 2.0, 2.0), 8.81 (1H, dd, *J*=5.0, 2.0 Hz), 9.00 (1H, dd, *J*=2.0, 1.0 Hz), 9.82 (1H, brs, exchangeable with D₂O). ¹³C-NMR (DMSO-*d*₆): δ : 62.6 (t), 69.4 (s), 123.9 (d), 124.8 (s), 126.6 (d), 128.3 (d), 128.5 (d), 136.8 (d), 139.0 (s), 149.9 (d), 153.4 (s), 154.0 (d), 163.5 (s), 172.4 (s). IR (Nujol): 1785, 1749, 1721, 1592, 1450, 1421, 1379, 1270, 1080 cm⁻¹. MS *m/z* (%): 387 (M⁺, 14), 251 (20), 236 (27), 208 (68), 180 (100), 165 (23), 124 (14), 106 (46). Anal. Calcd. for C₂₂H₁₇N₃O₄: C, 68.21; H, 4.42; N, 10.85. Found: C, 68.19; H, 4.50; N, 10.96. The chemical properties of this compound agreed perfectly with the values reported by Pop *et al.*⁶⁾

Determination of Lipophilicity 1) Partition Coefficient (PC) in *n*-Octanol/Water System: 0.1 N HCl or phosphate-buffered saline (pH 7.4) and *n*-octanol were saturated with each other before starting the experiments. DPH and its derivatives were dissolved in the *n*-octanol at indicated concentrations. The concentration of the compounds in both phases was determined according to the method reported previously.⁵⁾

2) Lipophilic Index by HPLC: DPH and its derivatives were dissolved in methanol. The mobile phase was a 0.03 M NH₄Cl-methanol (2:3, v/v) adjusted to pH 7.4. A 10 μ l quantity of sample was injected into the column (Inertsil ODS, 10 $\times$ 250 mm, GL Science Inc.) and the HPLC was operated at a flow rate of 1.0 ml/min and 25 °C. The eluate was monitored with a UV detector at a wavelength of 254 nm. The corresponding log *k'* values of the compounds were calculated using the following equation:

$$\log k' = \log \frac{(T_r - T_0)}{T_0}$$

where *T_r* is the retention time of DPH or its derivatives and *T₀* is that of formamide (*i.e.*, an unretained peak).

Measurement of p*K_a* The p*K_a* values of DPH and its derivatives were determined at concentrations ranging from 1.5 $\times$ 10⁻⁵ to 2.5 $\times$ 10⁻⁵ M by the spectrometric method of Albert and Serjeant at room temperature.⁷⁾

Determination of Solubility at Various pH Excess amounts (3 mg) of DPH and its derivatives were added to buffer solutions (3 ml) in the pH range of 3 to 10. The suspensions were incubated at 25 °C for 24 h with constant shaking, and were then filtered through a membrane filter (pore size, 0.45 μ m). To a filtrate (100 μ l), 40 μ l of the internal standard dissolved in acetonitrile was added, and the concentration of DPH or its derivatives in filtrates was determined under the same HPLC conditions as the PC experiment.

In Vivo Protein Binding to Rat Plasma Rats had free access to an MF diet (Oriental Yeast Co., Ltd.) and water before and during the experiment. The rats received DPH and its derivatives intravenously (20 mg/kg, DPH equivalent) *via* silicone tubing inserted into the jugular vein, and blood was taken from the tubing 30 min after administration of the drug. Plasma was obtained immediately by centrifugation and placed in an ultrafilter (micropartition system MPS-1, Amicon Co., Danvers, MA, U.S.A.), which was centrifuged at 3000 rpm for a period (approximately 20 min) sufficient to obtain an ultrafiltrate volume of approximately 20% of the initial sample. The contents of the compounds adsorbed on the membrane were negligible. A filtrate was added to the same volume of acetonitrile containing an internal standard (acemethacin, clonazepam or medazepam, 5 or 10 μ g/ml in acetonitrile), followed by centrifugation (15000 rpm). The concentrations of compounds in the supernatant and plasma were determined by HPLC.⁵⁾

In Vitro Binding Study to Rat Plasma Protein and BSA Rat plasma containing 1 mg/ml of EDTA was delipidated with butanol-diisopropyl ether.⁸⁾ The compounds were dissolved in ethanol, followed by dilution with the 0.067 M buffer to be less than 3% ethanol. In the binding study to rat plasma protein or BSA, the initial concentration of the compounds was in the range of about 50–350 μ M for DPH, 50–250 μ M for MNDPH, 50–250 μ M for VADPH and 50–200 μ M for VPDPH. BSA was dissolved at a concentration of 6.4 $\times$ 10⁻⁴ M in the same buffer. The aqueous solutions of the compounds were added to the same volume of BSA solution or delipidated rat plasma (18.4–21.6 mg protein/ml), and the mixture was incubated at 25 °C for 1 h with shaking. Samples were subjected to ultrafiltration (3000 rpm) at 25 °C for 1 min using an ultrafilter. Unbound compounds in the filtrates were determined by HPLC under the same conditions as described above. During this experiment, DPH, VADPH and VPDPH were found to be stable, but MNDPH in the delipidated rat plasma was quickly hydrolyzed, although this compound was almost stable in the BSA solution. Therefore, the binding experiment for MNDPH was not carried out.

In Vitro Hydrolysis Studies in Rat Tissues The rats were fasted overnight before sacrifice by decapitation. The liver was perfused through the portal vein with physiological

saline to remove blood. The small intestine contents were removed by flushing with saline, and the mucosa was scraped off with a stainless steel spatula. The cerebral cortex was rapidly removed and rinsed with saline. Each tissue was homogenized in 3 volumes of the next isotonic buffer: 0.01 M phosphate buffer (pH 7.4)–1.15% KCl for the mucosa and liver, and 0.01 M Tris–HCl buffer (pH 7.4) including 0.25 M sucrose and 1 mM EDTA for the cortex. After centrifugation of the homogenates, the resulting supernatants and plasma were diluted with 0.1 M phosphate buffer (pH 7.0 for the small intestine and pH 7.4 for the liver, plasma and cerebral cortex) to give 8 mg protein/ml. The protein concentrations were estimated by the procedure reported by Lowry *et al.* using BSA as a standard. For the measurement used with the plasma, mucosa and cortex, an aliquot (50 μ l) of the 1×10^{-3} M ethanol solutions of DPH and its derivatives was mixed with a 0.1 M phosphate buffer, pH 7.4 or 7.0, (750 μ l) supplemented with gentamicin sulfate solution. For the liver, a NADPH-generating system (25 mM MgCl_2 , 1 mM NADP, 25 mM G-6-P and 0.16 mM nicotinamide, 250 μ l) was added to the reaction mixture consisting of the buffer (pH 7.4, 500 μ l) and the compound solution (50 μ l). The reaction was initiated by adding the supernatant of each tissue. The mixture was incubated at 37 °C with constant shaking. At appropriate time intervals, a 0.1 ml aliquot was withdrawn and added to 2 volumes of acetonitrile containing internal standards. After mixing and centrifugation, an aliquot (50 μ l) of the upper fraction was injected onto the column, and the compound level was determined by the HPLC method with a minor modification: internal standard, acetaminophen (2 μ g/ml in acetonitrile) for DPH, VADPH and VPDPH, and indomethacin (2 μ g/ml in acetonitrile) for MNDPH; mobile phase, methanol–acetonitrile–0.025 M phosphate buffer (pH 7.0) (2:3:5, v/v) with 0.5 mM *tetra-n*-butylammonium phosphate (TBA-P), and without TBA-P for the determination of VPDPH.

Data Analysis for Protein Binding and Hydrolysis Experiments For the protein binding experiment, binding parameters were estimated by fitting the data to the following Scatchard and Rosenthal equations using a non-linear least-squares regression program (MULTI program).⁹⁾

Scatchard equation:

$$\frac{r}{C_f} = n \cdot K - K \cdot r$$

Rosenthal equation:

$$\frac{C_b}{C_f} = n \cdot P_t \cdot K - K \cdot C_b$$

where r and n are the number of moles of compound bound and the number of binding sites per mole of protein, respectively. K is the association constant. $n \cdot P_t$ in the Rosenthal equation is the binding capacity, and C_f and C_b are the concentrations of free and binding compounds, respectively.

In the hydrolysis experiment, the pseudo first-order rate constant (k_{obs}) was calculated from the following equation:

$$k_{\text{obs}} = \frac{2.303}{t} \cdot \log \frac{a}{a-x}$$

where a is the initial amount of the compound and x is the amount of DPH generated in time t . Significant differences were analyzed by Student's t -test, and the significance level adopted was $p < 0.05$.

RESULTS AND DISCUSSION

Physicochemical Properties It is well known that physicochemical properties, such as the lipid-water partition coefficient (lipophilicity of drugs) is associated with the ability of the drugs to cross the BBB.¹⁰⁾ The lipophilic index and $\text{p}K_a$ value of DPH and its derivatives containing MNDPH are summarized in Table I. Preliminary experiments confirmed that MNDPH was generally stable in aqueous solutions ranging from pH 3 to 8, and the other two derivatives were also stable in the pH range from 1 to 10. The $\log PC$ and $\log k'$ values for all derivatives were higher than those for DPH, with VPDPH having the highest lipophilicity. The plot of $\log PC$ versus $\log k'$ exhibited a straight line relationship. An optimum $\log PC$ value (octanol/water partition coefficient) in penetrating into the brain is assumed to be in the range of 1.5–2.5.¹¹⁾ In this study, the order of $\log PC$ value of the synthesized derivatives was VPDPH > VADPH > MNDPH > DPH. The $\log k'$ values of the derivatives were also increased in the same order.

The $\text{p}K_a$ value of DPH and its derivatives was measured by the spectrometric method.⁷⁾ As summarized in Table I, the values of the derivatives were much lower than that of DPH, suggesting that the derivatives would occur mostly in the ionized form at physiological pH. The solubilities of DPH, VADPH and VPDPH were very low and were kept nearly constant below pH 6 (0.94% for DPH, 0.07% for VADPH and 0.02% for VPDPH). In contrast, the solubility of MNDPH decreased with increasing pH but kept constant at pH 4–8 (0.10%). To clarify whether these derivatives were acidic or basic compounds, the data obtained in the pH-solubility studies were evaluated by the Henderson-Hasselbalch equation. The evaluation indicated that MNDPH was a basic compound and the other two derivatives were acidic.

It is of strong interest to estimate whether these chimera drugs, such as VADPH and VPDPH, have pharmacological activities, although this is now being studied.

In Vivo Binding to Rat Plasma Total concentrations

TABLE I. Physicochemical Properties of DPH and Its Derivatives

Compound	Partition coefficient (PC)		$\log k'^c$	$\text{p}K_a$
	$\log PC^a$	$\log PC^b$		
DPH	2.35	2.28	0.24	8.41
MNDPH	—	2.52	0.72	3.05 ^{d)}
VADPH	2.75	1.12	0.85	5.68
VPDPH	3.28	1.64	1.50	5.91

a) *n*-Octanol/0.1 N HCl (pH 1.0). b) *n*-Octanol/phosphate buffer (pH 7.4). c) Lipophilic index by the HPLC method. d) The $\text{p}K_a$ value measured in the pH range from 2.8 to 8.

TABLE II. *In Vivo* Binding of DPH and Its Derivatives to Rat Plasma Protein

Compound	Total concentration of drug in plasma ^{a)} ($\mu\text{g/ml}$)	Protein binding (bound %) ($\mu\text{g/ml}$)
DPH	15.5 ± 0.07	86.3 ± 1.7
MNDPH	— ^{b)}	— ^{b)}
VADPH	65.5 ± 3.4	98.7 ± 0.3
VPDPH	32.3 ± 4.1	99.1 ± 0.5

The values are the mean $\pm$ S.D. of 5–8 rats. Dose: 20 mg/kg (DPH equivalent).
 a) The sum of free and bound drug concentrations in rat plasma 30 min after i.v. injection. b) Not determined.

Fig. 2. Rosenthal Plots of DPH and Its Derivatives Binding to Rat Plasma Protein at 25 °C

○, DPH; △, VADPH; □, VPDPH. C_f , free compound concentration. C_b , bound compound concentration. The solid lines are theoretical curves computed using the binding parameters.

and the percent binding of each compound in plasma obtained at 30 min after i.v. administration are listed in Table II. Total concentrations of VADPH and VPDPH in plasma were 3.2 (195 μM) and 1.4 times (85 μM), respectively, higher than that of DPH (61 μM). By using an ultrafiltration method, both VADPH and VPDPH were found to be extensively bound to rat plasma. Consequently, the plasma free levels of VADPH and VPDPH decreased to only 1.3 (2.5 μM) and 0.9% (0.77 μM), respectively, in contrast to that of DPH (13.7%, 8.4 μM). Since MNDPH was quickly hydrolyzed in rat plasma, it was impossible to obtain data on binding to rat plasma. These results showed that the increase in log PC of the derivatives was related to the higher protein binding properties.

***In Vitro* Binding to Rat Plasma Protein or BSA** Figure 2 shows Rosenthal plots of the binding data of DPH and its derivatives to rat plasma at 25 °C. The Rosenthal plot for each binding gave a straight line, indicating that these drugs have a single binding site on the protein. The calculated parameters, an association constant (K) and the binding capacity ($n \cdot P_t$) are listed in Table III. The K values of VADPH and VPDPH were 16 and 81.5 times higher than that of DPH, respectively. This indicates that the binding strength of plasma protein for the derivatives is extensively higher than that of DPH, although the $n \cdot P_t$

TABLE III. Binding Parameters for DPH and Its Derivatives to Rat Plasma Protein at pH 7.4^{a)} and 25 °C

Compound	$n \cdot P_t$ ^{b)} (μM)	K ^{c)} (μM^{-1})
DPH	158.8 ± 0.05	0.053 ± 0.001
MNDPH	— ^{d)}	— ^{d)}
VADPH	168.3 ± 2.2	0.84 ± 0.02
VPDPH	162.9 ± 2.4	4.32 ± 0.23

Each value represents the computer-fitted parameter with S.D. The values were estimated from the Rosenthal plot using the non-least squares method. a) 0.067 M phosphate buffer. b) The binding capacity. c) The association constant. d) Not determined.

TABLE IV. Binding Parameters for DPH and Its Derivatives to BSA at pH 7.4^{a)} and 25 °C

Compound	n ^{b)}	K ^{c)} ($\times 10^4 \text{ M}^{-1}$)
DPH	1.00 ± 0.05	1.17 ± 0.07
MNDPH	0.38 ± 0.01	7.50 ± 0.48
VADPH	1.14 ± 0.05	11.7 ± 0.6
VPDPH	1.00 ± 0.04	18.2 ± 0.9

Each value represents the computer-fitted parameter with S.D. BSA concentration before this study was $6.4 \times 10^{-4} \text{ M}$. a) 0.067 M phosphate buffer. b) The number of binding sites. c) The association constant.

values of the derivatives were almost identical with that of DPH. The mean percent binding of DPH and the derivatives at the initial concentrations ranging from 50 to 200 μM were $89.1 \pm 3.9\%$ for DPH, $99.4 \pm 0.2\%$ for VADPH and $99.7 \pm 0.2\%$ for VPDPH, and these values were in good agreement with the data obtained by the *in vivo* binding study. The Scatchard plot for these drug bindings to rat plasma protein also showed a straight line, with highly similar K values (data not shown).

The binding parameters, calculated from the Scatchard plots, for the binding of DPH and its derivatives to BSA at 25 °C are summarized in Table IV. The association constants (K) for VADPH and VPDPH were also extremely high compared with that of DPH. These data suggest that the binding characteristics to BSA are similar to those to rat plasma protein. The mean percent binding of DPH, VADPH and VPDPH to BSA was 87.1 ± 1.2 , 98.5 ± 0.2 and $98.9 \pm 0.2\%$, respectively, at the initial concentrations ranging from 50 to 200 μM . These results were also consistent with the *in vivo* binding data. The Scatchard plot again showed that the respective drugs have a single binding site on BSA. Monks *et al.* reported that DPH bound to a single site on the human serum molecule.¹²⁾ The number of binding sites for MNDPH was less than one. Sjöholm *et al.* have reported that the number of binding sites of DPH on HSA is a single site, which is classified in the warfarin site group (site I).¹³⁾ In this study, the calculated binding site (n) of DPH and its derivatives on BSA was only one, which agreed well with the reported value. However, the number of binding sites of MNDPH was below one (Table IV). The possible reason involved in the small n value for MNDPH is unclear based on the present data.

As a result, the extraction ratio⁵⁾ of the derivatives by the brain correlated with neither the lipophilicity of the derivatives (Table I) nor *in vivo* protein binding (98.7 and

Fig. 3. Hydrolysis of MNDPH in Various Tissue Homogenates of Rat
 ●, plasma; ▲, small intestine; ■, liver; ◆, cortex. Each point represents the mean $\pm$ S.D. of 3–4 experiments. When the S.D. was small, it was included in the symbol. Each solid line was obtained by fitting a series of data.

99.1% for VADPH and VPDPH). Judging from the pK_a values of all derivatives, VADPH and VPDPH were found to be acidic compounds, while MNDPH was basic. There is a crucial point to be noted regarding the pK_a and the partition coefficient. Namely, under physiological conditions, most fractions of VADPH and VPDPH exist in an ionized form (the fraction existed in ionized form (F), 0.98 and 0.97, respectively). Their virtual $\log PC$ calculated at pH 7.4 (VADPH, 1.02; VPDPH, 1.78) were much lower than the $\log PC$ values (2.75 and 3.28, respectively, Table I), in contrast to both the $\log PC$ values (2.31 and 2.35, respectively) of DPH. Thus, the transport of these derivatives across the BBB would be extremely decreased as a result of the rapid elimination of their ionized fractions. Additionally, it is possible that the strong protein binding characteristics of VADPH and VPDPH might partly reduce the distribution into the brain.

In Vitro Hydrolysis of the Derivatives The hydrolytic conversion of the derivatives to DPH was studied using the small intestine, liver and brain tissues, as well as rat plasma. Only MNDPH was hydrolyzed in various tissues, and VADPH and VPDPH were not hydrolyzed, even when incubated over a period of 24 h. Figure 3 shows the semilog plots of the concentrations of remaining MNDPH as a function of time. The plots show good linearity, indicating that the reactions of MNDPH were adequately described by pseudo first-order kinetics. The pseudo first-order rate

constants calculated were $0.22 \pm 0.04 \text{ min}^{-1}$ in the plasma, $2.12 \pm 0.38 \text{ min}^{-1}$ in the small intestine, $0.94 \pm 0.04 \text{ min}^{-1}$ in the liver and $0.011 \pm 0.01 \text{ min}^{-1}$ in the brain, respectively. MNDPH was completely hydrolyzed within 10 min in the plasma, small intestine and liver and converted to DPH, whereas it was slowly hydrolyzed in brain, with a $t_{1/2}$ value of 63 min. Our result agreed well the data that MNDPH is hydrolyzed to DPH.¹⁴⁾

In conclusion, VADPH and VPDPH showed higher lipophilicities and acidic properties, being pK_a 5.68 and 5.91, respectively. Their virtual $\log PC$ values at physiological pH were much lower than that of DPH, and the derivatives mostly existed in an ionized form under physiological conditions. These derivatives were not converted to DPH in rat small intestine, plasma, liver or brain. They strongly bound to plasma protein, especially albumin, with a single binding site. In contrast, MNDPH was quickly converted to DPH in plasma and tissues, and the percent binding of MNDPH to BSA was only slightly higher than that of DPH. Consequently, low uptake of VADPH and VPDPH by the brain can be mainly attributed to both their high protein binding activity and the large dissociation of the derivatives in plasma, compared with that of DPH.

REFERENCES

- 1) P. K. M. Lunde., R. Anders, S. J. Yaffe, L. Lund, F. Sjoquist, *Clin. Pharmacol. Ther.*, **11**, 846 (1970).
- 2) W. M. Pardridge, E. M. Landow, *J. Clin. Invest.*, **74**, 745 (1984).
- 3) T. Lin, J. H. Lin, *J. Pharmacol. Exp. Ther.*, **253**, 45 (1990).
- 4) D. M. Woodbury, "Antiepileptic Drugs," ed. by D. M. Woodbury, J. K. Penry, C. E. Pippenger, Raven, New York, 1982, pp. 191–207.
- 5) T. Ogiso, M. Iwaki, T. Tanino, O. Muraoka, G. Tanabe, *Biol. Pharm. Bull.*, **16**, 1025 (1993).
- 6) E. Pop, E. Shek, T. Murakami, N. S. Bodor, *J. Pharm. Sci.*, **78**, 609 (1989).
- 7) S. Matsuura, "Ionic Constants-Sokuteiho To Ohyo," ed. by A. Albert, E. P. Serjeant, Maruzen, Tokyo, 1963, pp. 63–85.
- 8) B. Cham, B. R. Knowles, *J. Lipid Res.*, **17**, 176 (1976).
- 9) K. Yamaoka, T. Tanigawara, T. Nakagawa, T. Uno, *J. Pharmacobiodyn.*, **4**, 879 (1981).
- 10) T. Murakami, E. Shek, E. Pop, N. S. Bodor, *J. Pharm. Sci.*, **78**, 732 (1989).
- 11) H. N. Greig, *Cancer Treat. Rev.*, **14**, 1 (1987).
- 12) A. Monks, S. Boobis, J. Wadsworth, A. Richens, *Br. J. Clin. Pharmacol.*, **6**, 487 (1978).
- 13) I. Sjöholm, B. Ekman, A. Kober, I. Ljungstedt-Pahlman, B. Seiving, T. Sjödin, *Mol. Pharmacol.*, **16**, 767 (1979).
- 14) S. A. Varia, S. Schuller, V. J. Stella, *J. Pharm. Sci.*, **73**, 1074 (1984).