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Table II. Spiro[tetralin-1,3′-pyrrolidine] Derivatives 2a–n

compd	formula[a]	yield,[b] %	mp, °C	writhing test, mg/kg po	hot-plate test: ED_{50}, mg/kg po[g]
2a	$C_{13}H_{18}NCl$[c]	79	164–176	30, ++++	13.3 (10.5–15.7)
2b	$C_{14}H_{20}NCl$[c]	81	202–210	30, ++++	12.1 (8.4–14.3)
2c	$C_{17}H_{21}NO_5$[d]	85	182–189	30, ++	48.6 (30.3–57.6)
2d	$C_{18}H_{23}NO_5$[d]	92	132–136	100, +++	ND[f]
2e	$C_{14}H_{20}NOCl$[c]	90	174–192	100, +	ND[f]
2f	$C_{18}H_{23}NO_5$[d]	92	147–151	100, ++	ND[f]
2g	$C_{14}H_{20}NOCl$[c]	78	162–169	100, +	ND[f]
2h	$C_{18}H_{23}NO_5$[d]	85	129–135	30, ++	>50
2i	$C_{13}H_{18}NOBr$[e]	60	230–232	30, ++	>50
2j	$C_{14}H_{20}NOBr$[e]	88	255–259	100, +	ND[f]
2k	$C_{13}H_{18}NOBr$[e]	39	195–203	100, ++	ND[f]
2l	$C_{14}H_{20}NOBr$[e]	50	218–230	30, ++	>50
2m	$C_{13}H_{18}NOBr$[e]	67	258–261	100, ++	ND[f]
2n	$C_{14}H_{20}NOBr$[e]	52	182–193	30, ++	42.8 (35.6–49.4)
morphine hydrochloride				10, ++++	1.2 (0.9–1.5)
profadol				10, ++++	3.9 (2.5–5.9)

[a, b] See corresponding footnotes in Table I. [c] Isolated as the hydrochloride salt and recrystallized from ethanol–ether. [d] Isolated as the fumarate salt and recrystallized from ethanol. [e] Isolated as the hydrobromide salt and recrystallized from ethanol. [f] Not determined. [g] Confidence limits in parentheses.

Evaporation of the solvent afforded a yellow oil, which was distilled in vacuo to afford the appropriate spiro[tetralin-1,3′-pyrrolidine] as a colorless oil. Bases were converted immediately to their hydrochloride or fumarate salts.

Synthesis of *N*-Methylspiro[tetralin-1,3′-pyrrolidines]. The appropriate spiro[tetralin-1,3′-pyrrolidine] (1.1 g, 0.005 mol), HCO_2H (2.5 mL), and HCHO (37%, 1.0 mL) were heated together on a water bath for 7 h. After evaporation of the solution to dryness, the residual oil was dissolved in 5% HCl, washed with ether, basified with 10% NaOH, extracted with ether, and dried ($MgSO_4$). After evaporation of the solvent, the residual oil was distilled in vacuo to give the corresponding *N*-methyl derivative as a clear, colorless oil. The free base was converted to either the hydrochloride or fumarate salt (see Table II).

O-Demethylation of Compounds 2c–h. As a general procedure, the appropriate spiro[methoxytetralin-1,3′-pyrrolidine] (0.01 mol) was refluxed under nitrogen for 2 h at 125 °C in 48% aqueous hydrobromic acid (25 mL). The resulting yellow-brown solution was evaporated to dryness under nitrogen, and the residue was taken up in absolute ethanol. On addition of ether and refrigeration, buff crystals of the crude O-demethylated product were obtained. Recrystallization from ethanol–ether afforded a purer product (see Table II).

Pharmacology. Analgesia was determined by the acetic acid writhing test[15] in groups of six mice. Each group was dosed orally with either vehicle ("Dispersol") or compound under test and injected intraperitoneally 30 min later with dilute acetic acid (0.4 mL, 0.25%). The total number of writhes was recorded, and the protection afforded was expressed as a percentage of control values according to the following scale: ++++, 100% inhibition; +++, 75–99% inhibition; ++, 50–74% inhibition; +, 25–49% inhibition. Compounds showing 50% or more inhibition at 30 mg/kg in the above test were also tested for analgesia in mice by the hot-plate[16] method (see Table II).

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Relationship of Octanol/Water Partition Coefficient and Molecular Weight to Rat Brain Capillary Permeability

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The rat brain capillary permeability coefficient was determined for 27 compounds. The relationship of permeability to octanol/water partition coefficient and molecular weight was found to be predictable for drugs with molecular weights less than 400.

It is generally believed that the blood–brain barrier (BBB) is restrictive for small molecules at capillary endothelial cells and for large molecules at the interendothelial tight junctions. Although a great deal has been learned about the effects of BBB physiology on the passage of electrolytes and hydrophobic nonelectrolytes, a limited amount of information that correlates lipophilicity, molecular size, and the ability to cross the BBB has been published.[1–3]

We report the brain capillary permeability coefficient (P_c) determined in ether-anesthetized rats for 27 compounds for which the octanol/water partition coefficients are known.

Experimental Section

Isotopes. ^{14}C-labeled urea, creatinine, 5-fluorouracil, sodium ascorbate, and sucrose, ^{3}H-labeled water, glycerol, and galactitol, and $^{24}NaCl$ were purchased from New England Nuclear Corp. and/or Amersham-Searle, Inc. Radiopurity was satisfactory by manufacturer's specifications. ^{14}C-Labeled dianhydrogalactitol, dibromodulcitol, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea (PCNU), *N*-(1-methylethyl)-4-[(2-methylhydrazino)methyl]benzamide monohydrochloride (procarbazine), Baker's antifol, adriamycin,

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Table I. Rat Brain Capillary Permeability Coefficients (P_c)

compd no.	compd	N	M_r	log P	$P_c \times 10^{-6}$ cm/s
1	^3H_2O	13	18	−1.15	200
2	^{24}Na	9	58	−2.95	0.4
3	[^{14}C]urea	28	60	−2.80	0.82
4	[^{3}H]glycerol	10	92	−1.75	12
5	[^{14}C]creatinine	4	113	−1.77	0.28
6	5-fluoro[^{14}C]uracil	4	130	−0.95	1.7
7	[^{14}C]dianhydrogalactitol	6	150	−1.29	2.5
8	[^{14}C]metronidazole	11	171	−0.16	14
9	[^{14}C]ascorbate	5	176	−4.04	1.3
10	[^{3}H]galactitol	9	182	−3.10	0.39
11	[^{14}C]misonidazole	5	185	−0.37	10
12	[^{14}C]ftorafur	7	200	−0.48	6.4
13	[^{14}C]BCNU	2	214	1.54	154
14	[^{14}C]procarbazine	4	221	0.06	19
15	[^{14}C]CCNU	3	234	2.83	100
16	[^{14}C]pyrimethamine	4	249	2.69	120
17	[^{14}C]PCNU	5	263	0.37	11
18	DDMP	6	269	2.82	150
19	[^{14}C]DDEP	2	284	3.19	110
20	[^{14}C]dibromodulcitol	7	308	−0.29	1.9
21	[^{14}C]spirohydantoin mustard	7	315	2.47	29
22	[^{14}C]sucrose	3	342	−3.67	0.12
23	Baker's [^{14}C]antifol	2	398	−2.46	0.18
24	[^{14}C]adriamycin	4	543	−0.10	<0.014
25	[^{3}H]epipodophylotoxin	14	657	2.80	0.20
26	[^{3}H]vincristine	12	825	2.80	0.64
27	bleomycin	4	1400	−3.3	<0.014

misonidazole, ftorafur, and spirohydantoin mustard, and [^{3}H]-vincristine were supplied by Dr. Robert Engle (Chemical Resources Section, National Cancer Institute). Radiopurity and chemical purity were determined before use by thin-layer chromatography (TLC). Adriamycin and vincristine were repurified by TLC immediately before use (98% radiopurity). [^{14}C]Metronidazole was generously supplied by Dr. Rothwell Polk (Searle Laboratories). Radiopurity exceeded 98% by TLC. [^{3}H]Epipodophylotoxin (VM-26) was generously supplied by Dr. R. Dorrien Ven (Sandoz, Inc.). A radiopurity of 98% was confirmed by TLC. [^{14}C]Pyrimethamine and 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-[^{14}C]methylpyrimidine ([^{14}C]DDMP) were generously supplied by Dr. Charles Nichol (Wellcome Research Laboratories).

Nonradioactive Compounds. Bleomycin was a gift of Dr. Stanley Crooke (Bristol Laboratories). Chemical quantification of bleomycin from plasma and tissue samples was performed by Dr. James Strong (Baylor College of Medicine, Houston).[4]

DDEP [2,4-diamino-5-(3′,4′-dichlorophenyl)-6-ethylpyrimidine] was supplied by Dr. Charles Nichol (Wellcome Research Laboratories). Chemical quantification of plasma and tissue levels of DDEP were performed by Ellen Levin.[5]

Octanol/Water Partition Coefficients. The value of spirohydantoin mustard was calculated using π constants.[6] Partition coefficients for [^{3}H]glycerol, [^{14}C]creatinine, and ^{57}Co-bleomycin were determined in our laboratories using the techniques of Hansch.[7] Other values have been published.[8]

Brain Capillary Permeability Measurements.[2,9] For capillary permeability measurements, male Fisher 344 rats weighing 160 to 220 g were anesthetized with ether; isotopes were injected intravenously and blood samples were taken from the femoral artery at different times up to 6 min (to calculate the plasma drug or tracer integral). Rats were sacrificed by decapitation, and the heads were immersed in liquid nitrogen for 45 s. The brain was removed and both cortical and subcortical tissue sections were taken; care was exercised not to include large cerebral vasculature. For radioactivity measurements, tissue and plasma samples were placed in tared scintillation vials, reweighed, and digested with a tissue solubilizer, after which a toluene base fluor was added. For chemical analysis, tissue was placed into tared vials, reweighed, and frozen at −60 °C until analyzed.

The formula used to compute the capillary permeability coefficient, P_c, is shown in eq 1,[2,9] where the tracer distribution,

$$P_c = (DS/t)0.28(ICD)(BV)^{-1/2} \quad (1)$$

DS, over time in seconds is as shown in eq 2. In eq 2, C = cpm/g

$$DS/t = 0.93[C - (C)(PW)](AUC)^{-1/2} \quad (2)$$

of tissue, AUC (the area under the plasma curve during the experimental period) = cpm·min/g of plasma, PW = fractional tissue plasma water volume (mL/g), ICD = the intercapillary distance (cm), and BV = the fractional brain blood volume (mL/g). Values for PW, ICD, and BV for rat brain were determined previously.[2]

Results and Discussion

If the mechanism of nonelectrolyte permeation through capillary endothelial cells is similar to permeation into bulk lipid phases,

$$P_c \propto KD \quad (3)$$

where K is the membrane/water or lipid/water partition coefficient and D is the diffusion coefficient.[10] Because the relationship of the diffusion coefficient to molecular weight, M_r, in bulk solvents for small molecules (M_r < 1000) is[11,12]

$$D(M_r)^{-1/2} \approx \text{constant} \quad (4)$$

it follows that

$$P_c \propto K(M_r)^{-1/2} \quad (5)$$

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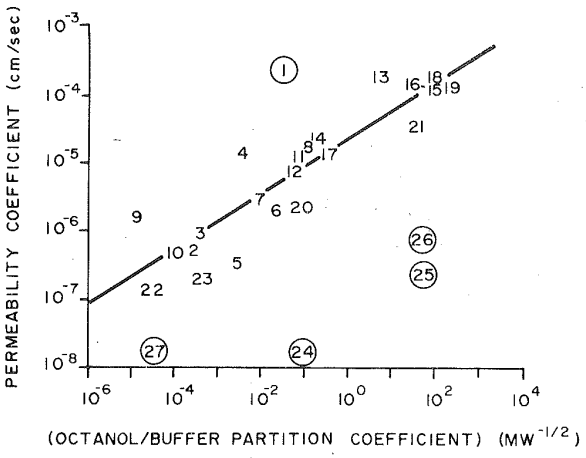

Figure 1. A plot of $P(M_r)^{-1/2}$ vs. P_c for the 27 compounds studied, fit by the method of least squares. The data are plotted as numbers that correspond to the list in Table I. Circled values were not used to compute the best fit line.

This conclusion has been reached by others,[10,11,12] although Collander concluded that eq 6 was a better fit for compounds with molecular weights between 70 and 480.[13]

$$P_c \propto (K_{oil})^{1.32}(M_r)^{-1.5} \quad (6)$$

As a reasonable and first approximation, we evaluated the relationship of P_c to $P(M_r)^{-1/2}$, where P is the octanol/water partition coefficient. Table I lists the molecular weight, log P, and P_c used in this analysis. Figure 1 is a plot of P_c vs. $P(M_r)^{-1/2}$. The line was fit by the method of least squares to 22 of 27 data points. For molecular weights below 400, the line in Figure 1 was fit to eq 7 with

$$\log P_c = -4.605 + 0.4115 \log[P(M_r)^{-1/2}] \quad (7)$$

an SE of estimate = 0.0431, SD of slope = 0.0423, $r = 0.91$, and $n = 22$. Of the five molecules not included, four (bleomycin, adriamycin, vincristine, and epipodophylotoxin) have molecular weights greater than 400, are extremely restricted in their ability to cross the BBB, and are considered to be excluded molecules.[2,9,14] Tritiated water was not included because other factors influence its membrane permeation.

The fact that epipodophyllotoxin and vincristine have permeability coefficients of 2.0×10^{-7} and 6.4×10^{-7} cm/s, respectively, yet are among drugs that do not cross the BBB[9,15] can be rationalized in two ways. First, the radioimpurities associated with these drugs may be smaller, and the more polar impurities may cross brain capillaries to a greater extent than the parent compounds. Second, because of high log P values (2.8), these drugs may penetrate and distribute into but not through brain capillary endothelia. In both cases, this would amount to a small net flux sufficient to produce the observed permeability. We support the second hypothesis because uptake of these labeled compounds over several hours did not indicate significant levels in rat brain.[14,15]

While it would be useful to compare a homologous series of compounds with different log P and molecular weights, it was not possible to do so. Only commercial radiolabeled molecules and labeled anticancer drugs supplied by the Chemical Resources Section, Division of Cancer Treatment, National Cancer Institute, were available to us. Nevertheless, the data are sufficient to derive useful insight into the physical criteria for passive BBB transport.

We draw two conclusions from this study. First, below a molecular weight of 400, increasing lipophilicity will improve P_c. For example, a molecule with $M_r = 400$ and $P = 1$ has a calculated P_c of 7.2×10^{-6} cm/s; for the same molecular weight and $P = 100$, however, P_c increases by nearly sevenfold to 4.8×10^{-5} cm/s. Second, although the absolute cutoff for "significant" BBB passage—regardless of lipophilicity—cannot be stated with certainty from the current study, it is clearly above 400 and below 657 daltons.

These studies have important implications for the design of psychotropic drugs, anticonvulsants, and brain tumor chemotherapeutic agents. The ability of an anticancer drug to cross the BBB is empirically associated with increased activity against CNS tumors when compared with like compounds that do not have this ability. BBB passage alone, however, is an insufficient criterion for antitumor activity. Plasma pharmacokinetics, rate and site of drug biotransformation, tumor capillary to cell transport and blood flow, and the mode of action of a drug are some of the factors that will modulate CNS antitumor activity.[16] Clearly, a logical step to developing better therapy and new therapeutic agents will be an understanding of physical transport factors, such as molecular size and lipophilicity, that influence brain capillary permeability.

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