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DRUG-INDUCED PORPHYRIN BIOSYNTHESIS—XIII

ROLE OF LIPOPHILICITY IN DETERMINING PORPHYRIN-INDUCING ACTIVITY OF ALIPHATIC AMIDES AFTER BLOCKADE OF THEIR HYDROLYSIS BY BIS-[*p*-NITROPHENYL]PHOSPHATE*

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Abstract—Hexanamide and heptanamide were the best substrates for a chick embryo liver amidase, and activity decreased with shorter or longer chain lengths. Amides branched at the 2-carbon atom were poor substrates and those branched at the 2- and 3-carbon atom were not hydrolyzed. Hydrolysis of both straight- and branched-chain amides was inhibited to varying extents by bis-[*p*-nitrophenyl]phosphate (BNPP). Branched-chain amides, while poor substrates of the amidase, were hydrolyzed solely by a BNPP-sensitive enzyme, while the straight-chain amides were hydrolyzed by a BNPP-sensitive and a BNPP-resistant enzyme.

The porphyrin-inducing activity of a series of aliphatic amides was studied in a chick embryo liver cell culture system in the presence and absence of BNPP. The potency of sterically hindered branched-chain aliphatic amides was found to be the same in the presence and absence of BNPP and could be correlated with lipophilicity. However, sterically unhindered aliphatic amides which were moderately potent in the absence of BNPP exhibited an increase in potency in the presence of BNPP. The potency of the straight-chain aliphatic amides after BNPP pretreatment could be correlated with lipophilicity. It was concluded that porphyrin-inducing activity of aliphatic amides in chick embryo liver cell culture depends upon lipophilicity and resistance to rapid metabolism to compounds of lower lipophilicity.

Studies of the relationship between chemical structure and porphyrin-inducing activity in rabbits [1] and in chick embryo liver cell cultures [2, 3] have shown that a series of amides are potent as porphyrin-inducing agents, while the corresponding acids are devoid of activity. For example, the amide, allylisopropylacetamide (AIA), is a potent porphyrin-inducing drug, while the corresponding free acid is inactive. Studies using cycloheximide and actinomycin D provide evidence for the fact that porphyrin accumulation in response to drugs results from induction of δ-aminolevulinic acid (ALA) synthetase [4, 5]. Recent evidence suggests that, in order for a chemical to induce porphyrin accumulation, it must remain in the liver for a period of at least several hr in order to induce and maintain high levels of ALA synthetase [6–9]. Consequently, it follows that a porphyrin-inducing drug should possess, in addition to other features, appropriate chemical properties that prevent it from being rapidly metabolized and inactivated by the liver. In previous studies [3], we have noted that aliphatic amides, such as allylisopropylacetamide, which are sterically hindered from hydrolysis by chick embryo liver amidases, possess potent porphyrin-inducing activity. On the other hand, aliphatic amides, such as pentanamide, which are not sterically hindered from hydrolysis, are devoid of porphyrin-inducing activity. However, it was surprising that 2-methylbutyramide, which was devoid of porphyrin-inducing activity, was nevertheless a poor substrate for the liver amidase. This observation showed that activity in this series of compounds is not explained completely on the basis of resistance to hydrolysis by the liver amidase and that other factors must contribute. The question then arises as to which other factors contribute to porphyrin-inducing activity [3]. De Matteis [10] studied the porphyrin-inducing activity of a series of amides and concluded that in chick embryo liver cell culture activity could be correlated with lipophilicity. In our studies, we have shown that lipophilicity alone is insufficient to explain porphyrin-inducing activity. For example, decanamide which has higher lipophilicity than allylisopropylacetamide is considerably less potent as a porphyrin-inducing drug. In a recent study [11] of the porphyrin-inducing activity of a series of aromatic esters, we have shown that porphyrin-inducing activity could be correlated with two properties of the esters, viz. lipophilicity and resistance to rapid hydrolysis to compounds of lower lipophilicity.

Heymann and Krisch [12] and Heymann *et al.* [13] showed that bis-[*p*-nitrophenyl]phosphate (BNPP) is a specific, relatively non-toxic inhibitor of liver carboxylesterase (carboxylic-ester hydrolase, EC 3.1.1.1.). The liver carboxylesterase also possesses amidase activity [14–17], and the hydrolysis of several amides can be inhibited by BNPP [13]. It appeared that the hydrolysis of sterically unhindered aliphatic amides might be inhibited by BNPP, thus prolonging the contact of unchanged drug with liver cells. Moreover, based on our experience with esters, it appeared of interest to determine whether the porphyrin-inducing activity of aliphatic amides could be correlated with lipophilicity, once hydrolysis was blocked. The objectives of this study were therefore: (1) to study the substrate specificity of chick embryo liver amidase; (2) to

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determine the effect of BNPP on the hydrolysis of a series of sterically hindered and unhindered aliphatic amides; (3) to determine whether inhibition of hydrolysis could be correlated with higher levels of unchanged drug in contact with liver cells; (4) to determine whether porphyrin-inducing activity of sterically unhindered amides would be increased after BNPP inhibition of their hydrolysis, and (5) to determine whether porphyrin-inducing activity could be correlated with lipophilicity after blockade of hydrolysis.

EXPERIMENTAL

Fertilized eggs of a white Leghorn strain were incubated at 38° at a relative humidity of 68 per cent.

Source of compounds and reagents. BNPP was prepared by the procedure of Corby *et al.* [18]. Allylisopropylacetamide (AIA) was supplied by Hoffmann-La Roche, Montreal, and AIA-2-^{14}C was obtained through the courtesy of Hoffmann-La Roche, Basel, Switzerland. Propylisopropylacetamide (PIA) and PIA-2-^{14}C were prepared from AIA [19]. *n*-Decanoic acid-1-^{14}C was purchased from Amersham/Searle. The appropriate acid or acid-chloride was converted to the corresponding amide by methods described previously [2]. Pooled bovine serum (lot 35) was purchased from Pentax, Inc., Winley–Morris Co., Ltd.

Determination of chick embryo liver amidase activity [3]. Livers from 17- and 18-day-old chick embryos were removed, homogenized in ice-cold 1·15% KCl (4 ml/g of liver) and centrifuged at 9000 *g* and 4° for 20 min. Aliquots (0·5 ml) of 1·0 M potassium phosphate buffer, pH 9·6, were added to approximately ten 1·0-ml portions of the supernatant in test tubes. To half of the test tubes was added BNPP (500 μg) in phosphate buffer (0·25 ml). To the other half was added phosphate buffer alone (0·25 ml). After a 1-hr period of incubation at 37°, solutions of aliphatic amides (17 μmoles) in phosphate buffer (0·25 ml) were added and the test tubes returned to the water bath for a further 7 hr. The ammonia content of the mixture was determined with the microdiffusion apparatus developed by Conway [20] and modified by Öbrink [21].

Cell culture techniques. Chick embryo liver cell cultures were prepared by using the procedure of Granick [4] with minor modifications [5]. After 24 hr of incubation, the media were removed from the cells and replaced with fresh media.

BNPP (50 μg) in 95% redistilled ethanol (5 μl) or ethanol alone (5 μl) was added to the cell cultures which were then returned to the incubator for 1 hr. Chemicals dissolved in ethanol (10 μl) were then added to the cell cultures and the cultures were reincubated. The porphyrin and protein content of the cells and medium were measured 24 hr later [4].

Extraction of ^{14}C-labeled drugs (decanamide, AIA and PIA) from cell culture medium. The procedure was that employed by Racz and Moffat [9]. A weighed amount of 1-^{14}C-decanamide was diluted with unlabeled drug to give material of specific activity, 0·007 μCi/μmole. This was dissolved in ethanol (10 μl) and added to cell cultures, which had been preincubated for 1 hr with either BNPP in ethanol (50 μg in 5 μl) or ethanol alone (5 μl), to give a concentration of 30 μg/ml of medium. The cell cultures were re-incubated, and after various periods, the media were collected and calcium- and magnesium-free Earle's solution was added to the cells. Five min later the Earle's solution was removed and 0·1 ml of 1 N NaOH was added to the combined medium and calcium- and magnesium-free Earle's solution in a separatory funnel. The aqueous phase was extracted with two 10-ml portions of ether. One ml of the ether extract was added to 10 ml toluene scintillation solution [consisting of 6 g of 2,5 diphenyloxazole (PPO) and 100 mg of 1,4-bis-2-(5 phenyloxazole)benzene (POPOP) in 1 liter of toluene] and counted. One ml of the aqueous phase was added to 10 ml Aquasol (New England Nuclear Corp.) and counted.

AIA-2-^{14}C (0·0146 μCi/μmole) and PIA-2-^{14}C (0·0203 μCi/μmole) in ethanol (5 μl) were added to the cell cultures to give a concentration of 7 μg/ml of medium. After various periods of incubation, the media were collected and calcium- and magnesium-free Earle's solution (2 ml) was added to the cells for 5 min. The combined medium and calcium- and magnesium-free Earle's solution were added to separatory funnels, saturated with sodium chloride and extracted with two 10-ml portions of ether. Two ml of the ether extract (20 ml) was added to 10 ml toluene scintillation solution.

The extraction for amides was checked by adding known amounts to medium alone. The ether extracts were found to contain 98·1 per cent of ^{14}C-AIA, 98·4 per cent of ^{14}C-PIA and 91·4 per cent of the 1-^{14}C-decanamide initially added. When 1-^{14}C-decanamide was incubated with medium alone at 37° for 4 hr, no hydrolysis was detected.

The ether extracts from all time periods were evaporated to dryness, the residues re-dissolved in 0·3 ml methanol and spotted as bands on 5 × 20 cm thin-layer plates coated with a 250 micron layer of Silica gel G. The plates on which decanamide was applied were developed with ammonia–methanol (1·5:100). The R_f of decanamide in this system was 0·68. The plates on which AIA and PIA were applied were developed with benzene–ethyl acetate (2:3). The R_f values of both AIA and PIA in this system were 0·35 [8]. The percentage of unchanged drug in the ether extracts was determined by the above thin-layer chromatographic method.

Determination of partition coefficients of AIA and PIA. Allylisopropylacetamide-2-^{14}C (0·184 mg, 231,000 dis/min/mg) in ethanol (50 μl) was added to a mixture of *n*-octanol (5 ml) and distilled water (15 ml), pH 6·8, in a separatory funnel. The contents of the funnel were thoroughly shaken and then centrifuged to separate the layers. Two ml of the *n*-octanol phase and five ml of the aqueous phase were added to Aquasol (10 ml) and then counted. The experiment was repeated using PIA instead of AIA.

Radioactive samples were counted in a Nuclear Chicago Mark II liquid scintillation system using an external standard channels ratio method to correct for quenching.

RESULTS AND DISCUSSION

Our first objective was to study the substrate specificity of a chick embryo liver amidase preparation. Schneck and Marks [3] measured the degree of hydrolysis of several aliphatic amides. This study has been extended and the results are shown in Table 1.

Table 1. Hydrolysis of a series of amides by chick embryo liver amidase before and after BNPP pretreatment

Compound	Enzyme activity* − BNPP	Enzyme activity* + BNPP	% Inhibition by BNPP
Acetamide	0·13 ± 0·08		
Pentanamide	22·3 ± 0·3 (8)	4·0 ± 0·7 (5)†	82·0
Hexanamide	41·8 ± 0·6 (7)	10·8 ± 0·8 (5)†	74·2
Heptanamide	35·4 ± 1·9 (4)	19·4 ± 1·6 (4)†	45·2
Octanamide	25·3 ± 0·6 (4)	17·6 ± 1·6 (5)†	30·4
Nonanamide	18·6 ± 0·4 (3)	11·0 ± 0·6 (3)†	40·9
Decanamide	16·1 ± 1·3 (4)	12·3 ± 0·7 (5)†	23·6
2-Methylbutyramide	7·9 ± 0·9 (5)	0·6 ± 0·2 (4)†	92·4
2-Ethylbutyramide	5·3 ± 0·3 (4)	0·4 ± 0·2 (4)†	92·5
Dipropylacetamide	5·3 ± 1·1 (4)	0·5 ± 0·2 (5)†	90·6
Allylisopropylacetamide	0·22 ± 0·03 (6)		
Propylisopropylacetamide	0·20 ± 0·12 (3)		

* The amount of amide (in μmoles) hydrolyzed by 9000 *g* supernatant from 1 g liver/7 hr. Values shown are the mean ± S.E. of the number of observations shown in parentheses.

† Denotes significantly lower values at 0·05 level when compared to amidase preparation which was not pretreated with BNPP.

Hexanamide and heptanamide were the best substrates, and activity decreased with shorter or longer chain lengths. These results are similar to those reported by Bray *et al.* [22], who studied rabbit liver amidase. Amides branched at the 2-carbon atom, viz. 2-methylbutyramide, 2-ethylbutyramide and dipropylacetamide, were poorer substrates of the amidase. On the other hand, amides branched at both the 2- and 3-carbon atoms, viz. AIA and PIA, were not substrates for the amidase. These results are of interest in the light of studies by Newman [23], who stressed that branching at the 3-carbon was the most important factor in causing steric hindrance to hydrolysis of esters and amides by either acid or base. Our results indicate that branching at the 3-carbon atom is also significant in producing steric hindrance to enzymic hydrolysis.

Rat and pig liver carboxylesterase possesses amidase activity [14–17] which is inhibited by a relatively specific inhibitor, BNPP, and it is possible that a similar situation exists with chick embryo liver carboxylesterase. The second objective of this study was to determine the effect of BNPP on the hydrolysis of aliphatic amides. The results of this study (Table 1) showed inhibition by BNPP of the hydrolysis of both straight- and branched-chain amides. Since the hydrolysis of branched-chain amides was almost completely blocked by this inhibitor, it appears that these amides, while poor substrates of the liver carboxylesterase, are nevertheless hydrolyzed solely by this BNPP-sensitive enzyme. In contrast, a BNPP-resistant hydrolysis of the straight-chain amides was observed which varied from 18·0 per cent with pentanamide to 76·4 per cent with decanamide. This observation suggests that, in addition to liver carboxylesterase, a second liver enzyme is involved in the hydrolysis of straight-chain aliphatic amides. The involvement of at least two enzymes in amide hydrolysis by rat liver has been reported by Heymann *et al.* [13].

Our third objective was to determine whether pretreatment of chick embryo liver cell cultures with BNPP would result in higher levels of unchanged amide in contact with the cell cultures. Decanamide-1-^{14}C was selected for study and was added to chick embryo liver cells in culture. At various time periods (0, 0·5, 1·5, 4·5 and 12 hr), the unchanged drug and metabolite(s) were separated by a solvent partition procedure. The results (Fig. 1) reveal that inhibition of hydrolysis of decanamide by BNPP results in significantly higher levels of unchanged drug in contact with the liver cells at the 1·5-, 4·5- and 12-hr time periods.

Since AIA and PIA were not hydrolyzed by the amidase, it was of interest to study the levels of these drugs in contact with liver cells at different periods of time. AIA-2-^{14}C and PIA-2-^{14}C were added to chick embryo liver cell cultures, and the percentage of unchanged drug was measured after 0, 6, 12 and 24 hr (Fig. 2). After 6 hr, 89 per cent of the AIA-2-^{14}C and

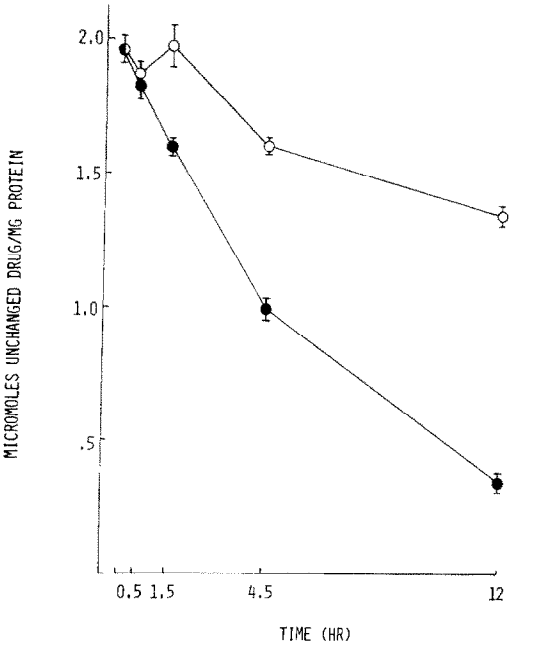

Fig. 1. Amount of unchanged decanamide-1-^{14}C found in the media after different periods of incubation of decanamide-1-^{14}C (30 μg/ml) with a monolayer of chick embryo liver cells without BNPP pretreatment (●——●) and with BNPP pretreatment (○——○).

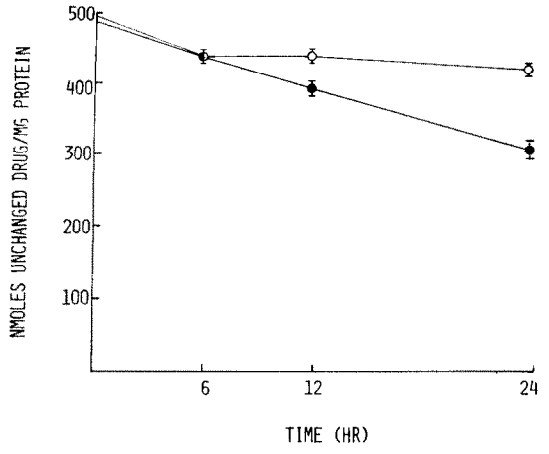

Fig. 2. Amount of unchanged AIA-2-^{14}C (O——O) and unchanged PIA-2-^{14}C (●——●) found in the media after different periods of incubation of AIA-2-^{14}C (7 μg/ml) and PIA-2-^{14}C (7 μg/ml) with a monolayer of chick embryo liver cells.

PIA-2-^{14}C, initially added to the medium, was recovered. Thereafter, there was a small but significant drop in the AIA-2-^{14}C level to 86 per cent at 24 hr. With PIA-2-^{14}C, the level dropped to 80 per cent at 12 hr and 62 per cent after 24 hr. These results are of interest for the following reasons. AIA is considerably more potent than PIA in inducing ALA synthetase in 17-day-old chick embryos, CBA/J mice [19] and rats [24]. The increased potency in chick embryos and mice is due at least in part to a slower rate of metabolism of AIA than PIA [8]. It has been claimed that AIA and PIA are approximately equipotent in chick embryo liver cell cultures [10, 19, 25]. We have reinvestigated the potency of AIA and PIA in this system, and our results (Table 2) show that PIA is considerably more potent than AIA. The high potency of PIA in chick embryo liver cell culture compared to other test systems was attributed [19] to the fact that, despite its more rapid rate of metabolism than AIA, the amount of PIA available to the cells is sufficient to ensure an effective concentration to stimulate ALA synthetase activity over the 24-hr period of incubation. The results in Fig. 2 showing that 62 per cent of the PIA is recoverable unchanged after 24 hr supports the hypothesis previously offered [19].

Our fourth objective was to determine whether the porphyrin-inducing activity of sterically unhindered amides would be increased after BNPP inhibition of their hydrolysis. This was found to be the case with hexanamide, heptanamide, octanamide, nonanamide, decanamide and dodecanamide (Table 2). On the other hand, pentanamide and 2-methylbutyramide remained inactive after BNPP pretreatment. The porphyrin-inducing activity of the sterically hindered amides, 2-ethylbutyramide and dipropylacetamide, remained unchanged in the presence of BNPP.

Our final objective was to attempt to correlate the porphyrin-inducing activity of the aliphatic amides with their lipophilicity after blockade of their hydrolysis with BNPP. Hansch *et al.* [26–28] have been able to correlate the relative biological activity of chemicals in a series of congeners with their lipophilicity. They have shown that the relationship between relative activity and lipophilicity can be either linear [26] or parabolic [27]. As a measure of lipophilicity, Hansch has used log P, where P is defined as the octanol–water partition coefficient of a drug. The log P values (Table 3) of AIA and PIA were obtained experimentally. The log P values of pentanamide and hexanamide were available [26, 27] and the log P values of other amides (Tables 3 and 4) were calculated by adding a value of 0·5 for each additional CH_2 group added to hexanamide [29]. In the case of branched-chain compounds, a value of 0·2 was subtracted from the calculated log P to compensate for branching [29]. Hansch has defined the relative activity of a drug in terms of log $1/C$, where C is the molar concentration of a drug producing a standard biological response. For our studies, we defined the porphyrin-inducing activity observed 24 hr after addition of AIA (10 μg/ml) to chick embryo liver cells as the standard biological response. Dose–response curves were constructed for the straight-chain amides in the presence of BNPP and for the branched-chain amides alone, and the molar concentration (C) was determined which gave the same response as AIA (10 μ/ml) in the same experiment.

The degree of correlation between log $1/C$ and log P was determined separately for the straight-chain amides in the presence of BNPP and for the branched-chain amides alone. For this purpose, regression analysis by the method of least squares was used to determine equations defining the "best" fit straight line (Equation 1) and the "best" fit parabola (Equation 2) through the data. For the straight-chain amides in the presence of BNPP, the equations derived were:

	n	r	S. D.	
$\log 1/C = 0{\cdot}560 \log P + 2{\cdot}341$	7	0·732	0·396	(1)
$\log 1/C = -0{\cdot}255 (\log P)^2 + 1{\cdot}589 \log P + 1{\cdot}626$	7	0·954	0·164	(2)

where n is the number of data points, r is the correlation coefficient and S. D. is the standard deviation [26, 27].

The log P value, the observed log $1/C$ value, the log $1/C$ value calculated from the parabolic equation (2) and the difference between log $1/C$ (obs'd) and log $1/C$ (calc'd) for each amide are shown in Table 4. The log P values were plotted against the observed log $1/C$ values in Fig. 3 and the parabola defined by Equation 2 was drawn through the data points. These results demonstrate that the porphyrin-inducing activity of the straight-chain amides after BNPP pretreatment can be correlated with lipophilicity and that the correlation is highest (r = 0·954) for the parabolic case (Equation2).

In the case of the branched-chain amides, the equations derived were:

	n	r	S. D.	
$\log 1/C = 0{\cdot}171 \log P + 1{\cdot}972$	5	0·970	0·189	(3)
$\log 1/C = -0{\cdot}640 (\log P)^2 + 2{\cdot}805 \log P + 1{\cdot}712$	5	0·996	0·070	(4)

Table 2. Porphyrin accumulation in a primary culture of chick embryo liver cells before and after treatment with bis-[*p*-nitrophenyl]phosphate

Compound*	Concn (μg/ml)	Porphyrin accumulation† (ng/mg protein) In absence of BNPP	In presence of BNPP (10 μg/ml)
Pentanamide	10	10·0 ± 1·1 (4)	9·3 ± 1·2 (5)
	30	6·7 ± 0·6 (5)	6·8 ± 0·4 (5)
	100	8·5 ± 0·4 (5)	6·1 ± 0·3 (5)
Hexanamide	10	7·7 ± 1·1 (8)	8·9 ± 0·8 (9)
	30	11·1 ± 2·0 (8)	9·3 ± 0·6 (8)
	100	36·0 ± 3·5 (4)	71·2 ± 13·5 (5)‡
Heptanamide	3	9·8 ± 1·1 (4)	10·3 ± 1·3 (4)
	10	17·2 ± 2·7 (4)	22·7 ± 2·8 (4)
	30	165·6 ± 39·9 (4)	486·8 ± 41·6 (8)‡
Octanamide	3	9·6 ± 1·1 (4)	11·1 ± 2·0 (4)
	10	18·0 ± 1·8 (4)	33·3 ± 12·2 (4)
	30	357·8 ± 80·0 (4)	1013·8 ± 49·3 (4)‡
Nonanamide	3	11·4 ± 1·3 (4)	12·0 ± 1·3 (4)
	10	10·7 ± 0·8 (4)	68·7 ± 13·3 (4)‡
	30	85·9 ± 20·7 (3)	1116·9 ± 77·5 (4)‡
Decanamide	3	9·5 ± 1·1 (4)	11·4 ± 1·0 (4)
	10	12·5 ± 2·4 (4)	66·3 ± 3·6 (4)‡
	30	33·5 ± 7·7 (4)	986·8 ± 75·1 (4)‡
Dodecanamide	10	23·6 ± 1·6 (4)	102·9 ± 4·4 (4)‡
	30	11·0 ± 5·7 (4)	719·5 ± 49·7 (4)‡
	60	593·9 ± 109·3 (4)	1044·8 ± 106·2 (4)‡
2-Methylbutyramide	10	5·9 ± 0·7 (4)	4·9 ± 0·4 (4)
	30	7·5 ± 0·7 (4)	6·6 ± 0·7 (4)
	100	9·4 ± 0·6 (4)	8·3 ± 0·2 (4)
2-Ethylbutyramide	10	7·5 ± 0·9 (4)	7·6 ± 0·8 (4)
	30	14·2 ± 3·0 (4)	16·5 ± 2·5 (4)
	100	233·6 ± 33·1 (4)	284·9 ± 14·0 (4)
AIA	5	32·8 ± 4·7 (6)	
	10	339·6 ± 28·3 (6)	
	15	347·7 ± 121·1 (5)	
PIA	5	307·8 ± 26·6 (6)	
	10	666·0 ± 27·7 (6)	
	15	765·0 ± 66·2 (6)	
Dipropylacetamide	3	545·9 ± 45·3 (4)	521·7 ± 88·3 (4)
	10	880·6 ± 71·7 (4)	805·7 ± 66·4 (4)

* Control experiments were carried out as follows: 95% ethanol (5 μl) was added to three dishes, and BNPP (50 μg) in 95% ethanol (5 μl) to three dishes. After 1 hr of incubation, 95% ethanol (10 μl) was added to the dishes which were reincubated for 24 hr. The porphyrin content of the dishes was then determined. The 95% ethanol control had 10·7 ± 0·9 ng/mg of protein and the BNPP–95% ethanol control 8·8 ± 0·2 ng/mg of protein.

† Values shown represent the mean of the number of determinations shown in parentheses ± S. E.

‡ Denotes significant difference at the 0·05 level.

Table 3. Observed and calculated concentrations of branched-chain aliphatic amides which give the same porphyrin-inducing activity as AIA (10 μg/ml).

$$\text{Log } 1/C = -0{\cdot}640\,(\log P)^2 + 2{\cdot}805 \log P + 1{\cdot}712 \quad \begin{matrix} n & r & \text{S.D.} \\ 5 & 0{\cdot}996 & 0{\cdot}070 \end{matrix} \quad (4)$$

Amide	Log *P*	Log 1/*C* Obs'd	Calc'd	ΔLog 1/*C*
2-Methylbutyramide	0·1	2·00	1·99	0·01
2-Ethylbutyramide	0·6	3·11	3·16	0·05
Allylisopropylacetamide	1·14	4·15	4·08	0·07
Propylisopropylacetamide	1·48	4·43	4·46	0·03
Dipropylacetamide	1·59	4·55	4·56	0·01

Table 4. Observed and calculated concentrations of straight-chain aliphatic amides which give the same porphyrin-inducing activity as AIA (10 μg/ml).

$$\text{Log } 1/C = -0{\cdot}255\,(\log P)^2 + 1{\cdot}589 \log P + 1{\cdot}626 \quad n = 7 \quad r = 0{\cdot}954 \quad \text{S.D.} = 0{\cdot}164 \quad (2)$$

Amide	Log P	Log $1/C$ Obs'd	Log $1/C$ Calc'd	ΔLog $1/C$
Pentanamide	0·29	2·00	2·07	0·07
Hexanamide	0·79	2·89	2·72	0·17
Heptanamide	1·29	3·06	3·25	0·19
Octanamide	1·79	3·74	3·65	0·09
Nonanamide	2·29	4·04	3·93	0·11
Decanamide	2·79	3·93	4·07	0·14
Dodecanamide	3·79	4·02	3·99	0·03

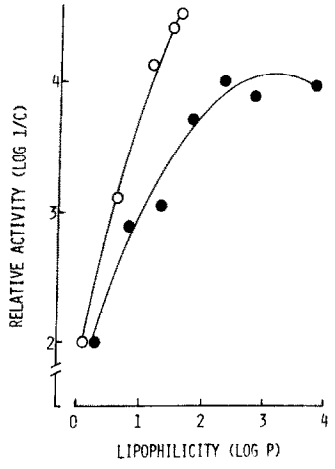

Fig. 3. Relationship between lipophilicity and porphyrin-inducing activity of straight-chain amides in the presence of BNPP (●), and of branched-chain amides alone (○).

The log P value, the experimentally observed log $1/C$ value, the log $1/C$ value calculated from the parabolic equation (4) and the difference between log $1/C$ (obs'd) and log $1/C$ (calc'd) for each amide are shown in Table 3. The log P values are plotted against the observed log $1/C$ values in Fig. 3 and the parabola defined by Equation 4 is drawn through the data points. The results demonstrate that the porphyrin-inducing activity of the branched-chain amides can also be correlated with lipophilicity; however, one is unable to determine whether the correlation is linear ($r = 0{\cdot}970$) or parabolic ($r = 0{\cdot}996$). A study of compounds in this series with higher log P values will be required before this question can be resolved.

Initially an attempt was made to correlate the porphyrin-inducing activity of all the amides together (the straight-chain amides in the presence of BNPP and the branched-chain amides alone) with their lipophilicity. Correlation coefficients of 0·393 for the linear and 0·698 for the parabolic case were obtained. The low correlation coefficients are most likely attributable to the fact that the branched-chain amides are virtually not hydrolyzed in the presence of BNPP, while the straight-chain amides undergo a BNPP-resistant hydrolysis (Table 1).

In summary, the activity of the straight-chain amides, with the exception of pentanamide, is increased after blockade of their hydrolysis by BNPP. The failure of BNPP pretreatment to increase the activity of pentanamide can be explained on the basis of the low lipophilicity of the compound. The activity of the branched-chain compounds is unchanged by BNPP. Furthermore, the inactivity of the branched-chain 2-methylbutyramide, which is relatively resistant to hydrolysis by the liver amidase, has now been shown to be due to its low lipophilicity. These results confirm the hypothesis that neither steric factors nor lipophilicity alone can explain porphyrin-inducing activity but that consideration of both these factors is required.

An important question that remains to be answered concerns the relevance of results obtained in chick embryo liver cells to results obtained in mammalian tests *in vivo* and in patients with hereditary porphyria. While a variety of drugs that precipitate acute attacks of porphyria in patients with the inherited trait are able to induce porphyrin accumulation in chick embryo liver cells [4], in the 17-day-old chick embryo [30] and in chickens [31], only a few exhibit activity in mice [30] or rats [32]. For this reason it has been suggested that results obtained using avian liver cells allow better prediction of results to be expected in porphyric patients than the results in mice [30], rats or rabbits [32]. There are two possible reasons for species variation in response to a drug [33]: (1) variation in drug metabolism resulting in differences in amounts of drug at the site of action, and (2) variation in sensitivity of receptor sites. Recent evidence suggests that both factors might be important. There is evidence for a partial block in heme biosynthesis in avian [34] and human porphyric liver cells [35], leading to increased sensitivity to the action of porphyrin-inducing drugs when compared to rat and mouse liver cells. Moreover, slow metabolism of drugs in chick embryo liver cells (Fig. 2) and in patients with acute intermittent porphyria [36] might in part explain increased sensitivity to these drugs.

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