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Rat Protein Binding and Cerebral Phospholipid Affinity of the H_3-receptor Antagonist Thioperamide

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Abstract

The binding of thioperamide, a known H_3-receptor antagonist, to rat plasma proteins and its affinity for rat cerebral phospholipids are investigated.

Thioperamide is strongly bound to plasma proteins (95–80% at plasma concentrations of 3·5–400 $\mu g\ mL^{-1}$), and its binding can be resolved into two components: a high-affinity, saturable component and a non-specific component. The drug has a high affinity for cerebral phospholipids, with a partition coefficient of approximately 100 ($\log K = 2{\cdot}06 \pm 0{\cdot}14$), which should promote brain penetration and accumulation.

Protein binding and cerebral phospholipid affinity can suggest the explanation of some differences reported in the literature on thioperamide distribution data: at low plasma concentrations of the drug, its protein binding (95% at 3·5 $\mu g\ mL^{-1}$) can prevent brain accumulation, while at higher concentrations the free plasma fraction suddenly increases (> 10% at 18 $\mu g\ mL^{-1}$) and it allows passive distribution to lipophilic tissues such as brain tissue.

Histamine receptors have been divided into three classes, named H_1, H_2 and H_3. Receptors of the last class, H_3, were initially described in rat cerebral cortex (Arrang et al 1983), where they inhibit the release and synthesis of histamine itself, acting as autoreceptors with presynaptic localization. Since 1983, H_3 receptors have also been observed in many other tissues, both in the central nervous system (CNS) and in peripheral organs, and they have exhibited a general regulatory behaviour on the release of histamine and other neurotransmitters (Schwartz et al 1990).

The variety of biological functions in which these receptors are involved makes the availability of potent and selective H_3-receptor agonists and antagonists a crucial point for the investigation of their physiological role. In particular H_3-receptor antagonists, which improve the release of histamine from nerve endings, could be useful to clarify the functions of histamine as a central neurotransmitter. The distribution of this kind of compound is obviously of great importance, most of all concerning the crossing of the blood-brain barrier and hence the possibility of reaching the CNS.

The H_3-receptor antagonist, thioperamide, has a potent ($pA_2 = 8{\cdot}96$) and selective action in-vitro, as reported for the first time by Arrang et al (1987), and it has also been employed to study its in-vivo effects on the CNS (Sakai et al 1991; Imaizumi & Onodera 1993; Yokoyama et al 1993; Clapham & Kilpatrick 1994; Malmberg-Aiello et al 1994; Owen et al 1994).

Bordi et al (1992a) presented the study of plasma and cerebral levels of thioperamide after intraperitoneal administration of 60 mg kg^{-1} to rats. It was observed that the drug had a wide distribution, as its initial plasma concentration was about 14 $\mu g\ mL^{-1}$ ($Vd = 4274$ mL kg^{-1}), and that it crossed the blood-brain barrier, with considerable amounts being found in the brain (about 8 μg/200 mg of lyophilized brain tissue, 60 min after administration) compared with plasma concentrations (approximately 11 mg mL^{-1} at the same time). Moreover, thioperamide showed long persistence in the blood, with a half-life of 10 h, while the cerebral levels, which increased during the first hour, decreased after 6 h although the drug was still present in the brain 12 h after administration.

Recently a study on the distribution of thioperamide in rats, at an intravenous dose of 10 mg kg^{-1} has been reported by Sakurai et al (1994); at that dose, thioperamide exhibited a rapid elimination, with a half-life of 30 min, and reached low levels in the brain compared with plasma concentrations (maximum brain concentration 0·2–1 $\mu g\ g^{-1}$ tissue in the different cerebral regions). The different experimental conditions consisted of different doses (60 and 10 mg kg^{-1}, respectively), routes of administration (intraperitoneal and intravenous) and rat weight (80 and 250 g). It appears that thioperamide reaches the CNS in considerable amounts at high doses, while it does not at lower doses.

The distribution and elimination of drugs is generally highly affected by protein binding (Audus et al 1992; Wagner 1993). Thus, we studied the binding of thioperamide in-vitro to rat plasma proteins, employing an ultracentrifugation technique, and we tried to gain some information on the nature of thioperamide protein binding, analysing the shape of the binding curve and fitting the data to some models derived from the classical Scatchard model.

We also considered whether thioperamide possessed the physico-chemical requirements to cross the blood-brain barrier. The importance of lipophilicity as a determinant of the ability to cross this barrier has long been recognized. This characteristic was first parametrized by the partition coefficient between *n*-octanol and water ($\log P_{oct}$) and Hansch et al (1987) reported a good number of drug series acting on the CNS which have shown parabolic relations between their potency and $\log P_{oct}$, with an optimal value of $\log P_{oct}$ around 2. Recently, more complex models have been considered, such as log P (log

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P_{oct} – log $P_{cyclohexane}$) presented by Young et al (1988), or a combination of solvatochromic parameters and a cavity term as described by Abraham et al (1994). According to these models, the H-bond acidity is one of the most important features in preventing the crossing of the blood-brain barrier. Besides these predictive models, related to linear free energy relationships or to linear solvation energy relationships, a more direct way to model the ability of a drug to cross a lipophilic barrier by passive diffusion is to measure its affinity for the cellular membranes themselves (Seydel et al 1992; Pauletti et al 1994). Unfortunately no reference model of this kind has been generally accepted so far, although some attempts at standardization have been made (Fan & Shen 1981; Pauletti et al 1994). In this context we developed a simple dialysis experiment to study the affinity of thioperamide for rat cerebral phospholipids, to have an indication as to its ability to dissolve into cerebral membranes and to accumulate in the cerebral lipophilic tissues.

Materials and Methods

Apparatus and chromatographic conditions

Thioperamide concentrations were measured by HPLC using a Gilson chromatograph equipped with a Rheodyne Model 7125 injector (20 μL). A Gilson Model 115 UV detector, working at a wavelength of 246 nm, was used.

The signal was recorded on a Hewlett-Packard Model HP 3994A recorder.

The chromatographic column was a prepacked Spherisorb ODS2 (Phase Separation, 250 × 4·6 mm, 10-μm particle size).

The mobile phase was a buffer at pH 7·4 with methanol (30 : 70); *n*-decylamine (0·2% v/v) was used as a masking agent to eliminate silanophilic interactions.

The aqueous portions of the mobile phase were prepared by first dissolving the buffering agent (3-morpholinopropanesulphonic acid, MOPS, 0·02 M) and the amino modifier (*n*-decylamine, 0·2 % v/v) in bi-distilled water and then adjusting the pH of this solution to the desired value by the addition of 0·2 M NaOH.

The areas were measured at room temperature; flow-rate was 1 mL min^{-1}.

Chemicals and reagents

Analytical grade methanol was obtained from C. Erba. 3-Morpholinopropanesulphonic acid (MOPS) was obtained from Fluka and *n*-decylamine (99%) from Aldrich Chemical Co. Thioperamide was synthesized in our laboratory according to Bordi et al (1992b).

Measurement of protein binding

Binding experiments. Thioperamide was added to plasma samples at different concentrations, ranging from 3·5 to 400 μg mL^{-1} (11·97–1368·00 μM). After 6 h of resting at 18°C, half of these samples were ultracentrifuged at 400 000 *g* for 6 h, while the others were taken as references.

Protein concentration was assayed with the Biorad method, using bovine serum albumin as a standard, and was found to be 80 mg mL^{-1}.

For the isolation of thioperamide in the ultracentrifuged portion, or in the reference samples, a solid-phase extraction technique was employed. One millilitre of methanol was passed through a Bond-Elut C18 cartridge (100 mg, Analytichem International), followed by 1 mL water (HPLC grade); the cartridge was then loaded with 1 mL sample, and rinsed with 1 mL water, under reduced pressure. Thioperamide was eluted with 2 mL methanol, and the volume of the eluate was adjusted to 5 mL with methanol. At least three samples of this solution (20 μL each) were injected in the chromatographic apparatus for thioperamide determination.

The concentration measured in the ultracentrifuged portion, corrected for recovery, was that of free thioperamide (F); the concentration of bound thioperamide (B) was calculated by subtracting F from the total concentration (C_{tot} from weighed amounts) of the drug, which was added to the plasma samples: $B = C_{tot} - F$.

The recovery of thioperamide was similar from whole plasma, ultracentrifuged plasma or water. Moreover, the percentage recovery was not significantly dependent on concentration in the range 3·5–400 μg mL^{-1} by one-way analysis of variance ($F_{(9,28)} = 1·949$, $P = 0·0855$, 10 groups, 38 measurements) or by Kruskal–Wallis analysis ($T = 11·79$, $P = 0·2256$); the mean recovery was 96·25 ± 2·963%. Statistical tests were performed with the statistical software Statgraphics 5·0 (STSC Inc).

The value of 96·25% was thus used to correct the concentrations measured on the samples of plasma after ultra centrifugation.

Calculation of protein binding curves. Binding curves were calculated according to a Scatchard model, fitting the data to the curve:

$$B_{calc} = \sum [B_{max,i} K_i F/(1 + K_i F)] \quad (1)$$

where the subscript i (from 1 to n) represents each class of binding site, considered as independent. Alternatively, a model which accounted for a non specific binding, in addition to a specific one, was employed, according to Taira & Terada (1985):

$$B_{calc} = \sum [B_{max,i} K_i F/(1 + K_i F)] + K_{asp} \quad (2)$$

The parameters of the curve were calculated by minimizing the squares of the residues $B - B_{calc}$, for all the 40 determinations. All the minimizations were performed with the Solver routine of the Spreadsheet Excel 4·0 (Microsoft Corporation).

Reversibility of protein binding. The reversibility of protein binding was evaluated by means of dialysis experiments in the following way. Thioperamide (at concentrations of 20 and 5 μg mL^{-1}) was added to a glass tube containing 10 mL aqueous buffer (EDTA 1 mM, NaCl 150 mM, phosphate buffer 20 mM) and its concentration was measured by HPLC. Into this tube a closed dialysis tube was placed, containing 1 mL plasma. After 24 h, the concentration of the drug in the external buffer solution was measured and the amount of thioperamide which diffused in the internal compartment was calculated. The dialysis tube was then removed, washed and introduced into a new tube containing 10 mL fresh buffer solution. After 24 h, the concentration of thioperamide which diffused to the external solution was measured. The binding of thioperamide to plasma proteins was reversible, since the amount bound decreased upon dilution of the expected amount from the second to the third stage. At these concentrations of thioperamide, no binding to the apparatus was observed.

Measurement of phospholipid affinity

Preparation of rat brain phospholipid suspension and dialysis experiments. Rat brain phospholipids were prepared by extracting lyophilized rat brains (Wistar rats, 250 g) with chloroform-methanol (1 : 1), evaporating the extract to dryness and suspending the residue in a buffer solution (EDTA 1 mM, NaCl 150 mM, phosphate buffer 20 mM). The suspension was sonicated in an ice bath for 15 min.

The dialysis apparatus employed for the study of the affinity of thioperamide was composed of a dialysis tube (cellulose, MW cut-off 5000, Wisking), closed at the bottom, containing 1 mL suspension of phospholipids (3% w/w) prepared as above. This dialysis tube was placed into a glass tube containing 10 mL buffer solution.

Thioperamide was added to the external compartment at various weighed initial concentrations, and the diffusion of the drug was allowed for 24 h. The thioperamide concentration in the external compartment was then measured, and the amount dissolved in the phospholipid micelles (Q_{PL}) was calculated considering the amount of the drug which remained in the external compartment, the amount which bound to the apparatus in an irreversible manner, and the amount which was dissolved in the volume of water in the internal compartment.

To determine the volume of water in the internal compartment (V_{int}) we measured the volume occupied by [^{3}H]inulin in parallel dialysis experiments (10 000 MW cut-off membrane), which gave $V_{int,w} = 0{\cdot}97$ mL.

The concentration of thioperamide in the lipophilic phase (C_{PL}) was calculated considering the volume of phospholipids (V_{PL}) as that excluded for [^{3}H]inulin (0·03 mL), although this included the internal water; the concentration thus calculated was therefore a lower limit of the concentration in the lipophilic phase only. The logarithm of the partition coefficient between phospholipids and water, log K, was calculated as:

$$\log_{10}(C_{PL}/C_{ext,fin}) \qquad (3)$$

Binding to the apparatus. To evaluate the amount of thioperamide binding to the dialysis apparatus, control experiments were performed employing dialysis tubes filled with buffer solution. Thioperamide in weighed amounts was dissolved in the external solution and allowed to diffuse into the dialysis tube. After 24 h this was washed and immersed in fresh buffer solution. Concentrations of the drug in the external compartment were measured at the beginning ($C_{ext,1}$), after 24 h ($C_{ext,2}$), and 24 h after the immersion of the loaded dialysis tube in fresh buffer solution ($C_{ext,3}$).

From the data represented in Fig. 1, we observed that, at initial concentrations higher than 40 μg mL^{-1}, there was a loss of thioperamide in the external compartment higher than expected according to the dilution. This was attributed to the binding of the drug to the experimental apparatus, which is usually encountered in dialysis experiments (Fois & Ashley 1991). This binding appears to be irreversible, as in the second stage of the experiment there is no further loss of drug; in fact $C_{ext,3}$ was not significantly different from the value expected from the dilution of the drug present in the internal buffer compartment at the beginning of this second stage.

The amount of thioperamide bound to the apparatus (Q_{app}, open circles in Fig. 1) is negligible at initial concentrations < 40 μg mL^{-1}, but at higher concentrations it depends in a linear manner on the initial concentrations according to the relation:

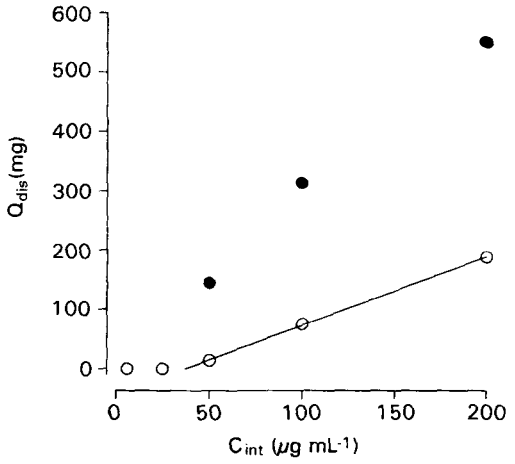

Fig. 1. Difference between the amount of thioperamide introduced in the external compartment of the dialysis apparatus and the amount calculated from the concentration found at the end of the experiment. ○ The amount of thioperamide which bound to the apparatus in control dialysis experiments, with a buffer solution in both the compartments. The line represents the equation $Q_{app} = 1{\cdot}172 \times (C_{ext,1} - 37{\cdot}030)$, used to calculate the amount bound to the apparatus at concentrations > 40 μg mL^{-1}. ● The amount of thioperamide which disappeared, after 24 h, when phospholipids were present in the internal compartment. The vertical distance from the line is the amount bound to phospholipids.

$$Q_{app} = 1{\cdot}172 \times (C_{ext,1} - 37{\cdot}030) \qquad (4)$$

where Q_{app} and $C_{ext,1}$ are expressed in μg and μg mL^{-1}, respectively. In the dialysis experiments, Q_{app} was therefore estimated by means of this equation.

Reversibility of phospholipid binding. To test the reversibility of thioperamide binding to phospholipids, we prepared a series of experiments as described above, except that phospholipids (3% w/w) were present in the dialysis tube.

Thioperamide was initially added to the external compartment at concentrations of about 50, 100 and 200 μg mL^{-1} (Table 1). At the end of the first diffusion stage (from the external to the internal compartment), in order to calculate the amount of thioperamide bound to phospholipids, the amount of the drug which apparently disappeared from the two aqueous compartments was calculated as:

$$Q_{dis} = [(C_{ext,1} - C_{ext,2}) \times V_{ext} - C_{int,2} \times V_{int}] \qquad (5)$$

where $C_{int,2}$ was set as equal to $C_{ext,2}$, $V_{ext} = 9{\cdot}85$ mL, and $V_{int} = 0{\cdot}97$ mL. These amounts are plotted against the initial concentration in Fig. 1, where the amounts bound to the apparatus are also shown. The difference between Q_{dis}, and the amount bound to the apparatus (calculated from the line shown in Fig. 1) gave the amount of drug bound to phospholipids. The concentration of thioperamide bound to phospholipids was therefore calculated as:

$$C_{PL,2} = [Q_{dis} - Q_{app}]/V_{PL} \qquad (6)$$

where $Q_{app} = 1{\cdot}172 \times (C_{ext,1} - 37{\cdot}030)$, as previously defined, and $V_{PL} = 0{\cdot}03$ mL.

To calculate the amount of thioperamide bound to phospholipids at the end of the second diffusion stage (from the

Table 1. Reversibility of thioperamide binding to phospholipids.

Parameter	Thioperamide (initial concn (μg mL^{-1}))		
	50	100	200
$C_{ext,1}$	49·039 ± 1·063	100·254 ± 0·870	194·387 ± 1·694
$C_{ext,2}$	34·422 ± 1·141	68·456 ± 5·215	138·464 ± 0·845
$C_{PL,2}$	3217 ± 102	5756 ± 1597	7737 ± 182
log K_1	1·971 ± 0·027	1·925 ± 0·158	1·747 ± 0·008
$C_{ext,3}$	7·293 ± 1·223	15·114 ± 0·852	25·498 ± 3·253
$C_{PL,3}$	1663·29 ± 373·15	2443 ± 1737	2890 ± 1376
log K_2	2·358 ± 0·171	2·209 ± 0·373	2·054 ± 0·276

internal to the external compartment), the amount of drug which diffused outside ($C_{ext,3} \times V_{ext,3}$ with $V_{ext,3} = 10$ mL) and that in the aqueous internal compartment ($C_{int,3} \times V_{int}$ with $C_{int,3} = C_{ext,3}$) were subtracted from the amount initially present in the whole internal compartment (aqueous buffer and phospholipids), calculated as:

$$(C_{ext,1} - C_{ext,2}) \times V_{ext} - Q_{app} \quad (7)$$

with V_{ext} and Q_{app} as previously defined.

As can be seen from Table 1, after the second diffusion stage, owing to the immersion of the loaded dialysis tubes in fresh buffer solution, the concentration of thioperamide bound to phospholipids ($C_{PL,3}$) falls to values which are about 50% Of $C_{PL,2}$. Therefore, the binding of thioperamide to phospholipids appears to be highly reversible, although the ratio between the concentrations of the drug in phospholipids and in water is slightly higher after the second diffusion stage than after the first (see log K_1 and log K_2 in Table 1).

Results

Protein binding

Mean values of free and bound concentration of thioperamide found in plasma samples at total concentrations ranging from 3·25 to 400 μg mL^{-1} are reported in Table 2. The same data are represented in Fig. 2 as a Scatchard plot. Seen on a large scale of free concentrations, the binding isotherm resembles that of a single binding site, and, as can be seen from Table 3, the models corresponding to a single aspecific site ($B = kF$) and to a single specific site ($B = B_{max}kF/(1 + kF)$) provide a satisfactory interpolation of the data. On the other hand, the Scatchard plot in Fig. 2 indicates the presence of two kinds of binding sites, the second being predominant at very low concentrations.

Table 2. Binding of thioperamide to plasma proteins.

Initial concn (μg mL^{-1})	Initial concn (μM) C_{tot}	n	Free concn (μM ± s.d.)	Bound (μM)	(%)
3·5	11·97	3	0·54 ± 0·07	11·42	95·46
6·5	22·23	2	1·39 ± 0·28	20·84	93·75
12·5	42·74	4	2·52 ± 0·35	40·23	94·12
18	61·55	4	6·86 ± 0·58	54·69	88·86
25	85·49	3	16·28 ± 0·58	69·20	80·95
50	170·98	4	36·49 ± 4·01	134·48	78·66
100	341·95	4	78·59 ± 9·45	263·36	77·02
200	683·90	8	146·49 ± 16·38	537·41	78·58
400	1368·00	8	280·22 ± 44·79	1087·58	79·51

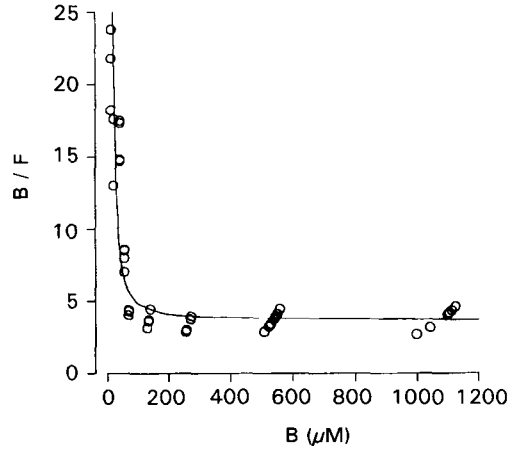

Fig. 2. Scatchard plot of the data on the binding of thioperamide to rat plasma proteins. Each circle represents an individual experiment, and the curve results from the fitting to a specific and non specific model.

For this reason we tried fitting a model with two specific binding sites, but the minimization of the sum of squares of errors led to a value of zero for $B_{max,2}$ (see experimental part), giving the same curve as the one-site model. This behaviour prompted us to employ the specific and non-specific binding model of Taira & Terada (1985), according to the equation:

$$B = B_{max,1}k_1F/(1 + k_1F) + k_{asp}F \quad (8)$$

where k_{asp} is the association constant of the binding to non-specific sites (Table 3). This model, although not significantly better than simpler ones as to the description of the data (a rigorous comparison is however difficult, given the unequal error distribution and the interdependence of the two variables), can provide an interpretation of the left branch of the Scatchard plot (Fig. 2).

Thus, binding of thioperamide to plasma proteins apparently takes place at two classes of sites, specific and non-specific, as reported for other drugs (Taira & Terada 1985; Wagner 1993). At low concentrations (up to 12·5 μg mL^{-1}) the specific binding predominates, while at higher concentrations the non-specific binding becomes more important. The percentage of bound thioperamide remains around 78% at high concentrations, where the relationship between B and F is linear, and increases to over 90% when total concentrations reduce to less than 12·5 μg mL^{-1} (Table 2).

Phospholipid affinity

The results of the dialysis studies on the affinity of thioperamide for cerebral phospholipids are reported in Table 4. As can be seen in Fig. 1, the amount of drug bound to phospholipids was considerably higher compared with that bound to the apparatus at initial concentrations of 50 μg mL^{-1} or more. At lower concentrations, the experimental error prevented an accurate estimate of the amount bound to phospholipids (data not shown). However, in the concentration range of 50–400 μg mL^{-1}, a roughly linear relationship between the calculated concentration in the phospholipids (C_{PL}) and the final concentration of thioperamide in water ($C_{ext,2}$) was observed. This is typical of a partition process, and the log of the apparent partition coefficient (log K in Table 4) was approximately 2 at all the different concentrations (2·06 ± 0·14). Thioperamide

Table 3. Binding constants for thioperamide to plasma proteins.

		Single specific site	Non-specific binding	Two specific sites	Specific and non-specific binding
k_1	(μM^{-1})	0·000835	3·729	0·000835	2·182
k_2	(μM^{-1})			*	3·612
$B_{max,1}$		5405·28		5405·128	25·295
$B_{max,2}$				0	
$\sum(B - B_{calc})^2$		341,284	390,080	341,284	379,465
Number of parameters		2	1	4	3
s.d. around model function	(μM)	94·769	100·010	97·366	101·271

*meaningless, as B = 0

Table 4. Affinity of thioperamide for rat brain phospholipids.

Initial concn ($C_{ext,1}$) ($\mu g\ mL^{-1}$)	24-h concn ($C_{ext,2}$) ($\mu g\ mL^{-1}$)	Concn in phospholipid (C_{PL}) ($\mu g\ mL^{-1}$)	log Kd
399·854 ± 0·885	249·699 ± 4·140	27804 ± 1483	2·047 ± 0·031
299·654 ± 0·268	204·761 ± 5·103	14751 ± 1828	1·858 ± 0·065
200·122 ± 0·068	114·416 ± 1·079	18498 ± 387	2·209 ± 0·013
150·331 ± 0·644	96·822 ± 7·393	10279 ± 2649	2·026 ± 0·160
99·839 ± 0·421	61·919 ± 1·745	8184 ± 625	2·121 ± 0·046
75·309 ± 0·158	44·517 ± 1·363	7329 ± 489	2·217 ± 0·043
50·043 ± 0·210	35·945 ± 2·193	3029 ± 786	1·926 ± 0·143

Data are means ± s.e.m. of three experiments for each concentration.

thus showed an affinity for phospholipids 100 times that for water.

Discussion

The curve of the binding of thioperamide to rat plasma protein suggests the presence of more than one kind of binding interaction, which is plausible given the heterogenicity of plasma proteins. In particular, the lack of a saturation level (which is a rather common feature for protein binding (Brodersen et al 1988), and the horizontal right arm of the Scatchard plot (Fig. 2) are probably related to a non-specific binding, which could be due to the hydrophobic nature of the drug. On the other hand, the left arm of the Scatchard plot and the initial curvature of the B versus F line (Fig. 2) reveal the presence of a saturable binding, especially evident at lower concentrations. The presence of specific and non-specific protein binding has been described in other cases, as for the binding of warfarin, salicylic acid, indomethacin (Taira & Terada 1985) and ibuprofen (Wagner 1993), and it causes a decrease in the free fraction of the drug at lower concentrations, which can explain some aspects of the reported pharmacokinetic behaviour of thioperamide.

A high affinity of thioperamide for cerebral phospholipids was also observed, described by a log K of 2·05 (Table 4). The composition and preparation methods of phospholipid vesicles strongly influence their molecular packing and fluidity, which are crucial for their interaction with drug molecules (Pauletti et al 1994). However, rather than looking for a highly standardized method, we examined the partition of thioperamide with a mixture of lipids whose composition resembled that of lipophilic rat cerebral tissues, to obtain a direct measurement of the affinity of the drug for these tissues, in spite of the lower reproducibility of the method.

It is noteworthy that the log K observed was very similar to the log $P_{o/w}$ of thioperamide (2.13, unpublished results), and that a log $P_{o/w}$ around 2 was indicated by Hansch et al (1987) as an optimal value for a drug acting on the CNS. In any case, given the high affinity of thioperamide for cerebral phospholipids, it appears that the lipophilic component of the blood-brain barrier cannot hinder the passive diffusion of thioperamide in the CNS.

The knowledge of the affinity of thioperamide for plasma proteins and for cerebral phospholipids can help us in the interpretation of the previous studies on the pharmacokinetics of thioperamide.

In the literature, different pharmacokinetic behaviour of this drug has been reported. There is a rapid elimination from the blood with low cerebral levels, after intravenous administration of 10 mg kg^{-1} to 250-g rats (Sakurai et al 1994), and a long half-life with good cerebral levels, for intraperitoneal administration of 60 mg kg^{-1} to 80-g rats (Bordi et al 1992a). The different weights (or ages) of rats can hardly explain these differences, since younger animals generally show faster elimination of drugs, while in this case they have a longer half-life for thioperamide. On the other hand, a 20-fold increment in half-life is probably not related to the different routes of administration only, since absorption after intraperitoneal administration is generally rather fast, and a first-pass metabolism cannot explain an increment in half-life. The dose of thioperamide administered seems therefore to be a determinant for its distribution.

One of the reasons for the apparently higher distribution of thioperamide in the brain at higher doses may be its lower percentage of molecules bound to plasma protein. According to the specific and non-specific binding model employing the parameters shown in Table 3, the percentage of free thioperamide in plasma is 2·2–2·5% at total concentrations of 2–

3 $\mu g\ mL^{-1}$ (6·84–10·26 μM), corresponding to the initial (15 min) concentrations with 10 $\mu g\ kg^{-1}$ (Sakurai et al 1994), while it is 10·1–11·4% at total concentrations of 12·5–14·5 $\mu g\ mL^{-1}$ (42·75–49·59 μM) observed at 15 min with 60 $\mu g\ kg^{-1}$ (Bordi et al 1992a).

Besides protein binding, which can influence the cerebral levels of thioperamide at the early stages of its diffusion, the half-life of the drug can also determine its maximum cerebral levels. In fact, while at a dose of 60 $\mu g\ kg^{-1}$ cerebral levels continue to increase for 180 min (Bordi et al 1992a), thanks to relatively high plasma concentrations, at 10 $\mu g\ mL^{-1}$ the plasma levels rapidly decrease (Sakurai et al 1994). This can prevent the reaching of high cerebral levels, in spite of the affinity for lipophilic tissues.

The elimination of thioperamide is another strongly dose-dependent feature of its pharmacokinetics. As the longest half-life corresponds to the highest free plasma fraction, the influence of protein binding on drug elimination can be ruled out. The differences in half-lives can instead be explained on the basis of the high binding of thioperamide to peripheral tissues. This wide diffusion of thioperamide has been observed by Sakurai et al (1994), measuring its levels in peripheral tissues, but it is even wider at a dose of 60 mg kg^{-1} (Bordi et al 1992a). At this dose a slow release from the loaded tissues to plasma can be supposed, assuming that the loading of the tissue would be much greater than at 10 mg kg^{-1}.

In conclusion, thioperamide seems to possess the lipophilic requirements for a good transfer across the blood-brain barrier, although the amount which actually reaches the brain depends on several dose-related factors, such as its protein binding and half-life. However, the apparently low levels recorded in the brain at lower concentrations can allow an in-vivo effect, given the very high potency of this drug. The high protein binding, while apparently not influencing the rate of elimination (faster at low doses, where the unbound fraction is lower), seems to prevent, at low doses, a free diffusion in the brain.

New H_3-receptor antagonists, which would exhibit a lower binding to plasma proteins, could perhaps have higher brain/plasma ratios, thus being useful for the study of the role of H_3-receptors in the CNS, with lower peripheral side-effects.

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