back to the value before freezing. That this only occurs for the ternary gel containing 0·5% w/w cetostearyl alcohol, a sample known not to contain any lyotropic liquid crystalline phase (Patel et al 1985) and that all the other samples exhibited hysteresis proportional to their cetostearyl alcohol concentration, would suggest that the very large increases in conductivity on thawing these samples must either be due to some breakdown of the molecular structure of the liquid crystalline phase and maybe release of some bound cetrimide into solution or a change in the tortuosity of the overall structure of the gel. The evidence from the differential interference microscopy would appear to support the latter mechanism. That the maxima in the hysteresis curves all occurred at the same temperature and that subsequent experiments have shown that this temperature varies with the batch and source of the cetostearyl alcohol used to prepare the gels and emulsions, would suggest that the decrease in conductivity after this point is due to the reaction between the cetrimide in solution and the excess cetostearyl alcohol reforming the liquid crystalline phase. Hence this characteristic temperature is analogous with the T_{pen} reported by previous workers (Lawrence 1959; Barry & Shotton 1968) except that in this case the cetostearyl alcohol is likely to be contaminated having been subjected to the processing involved in the preparation of the gels and emulsions.

Although this mechanism is somewhat speculative, it is supported by the observations on the viscosity of the samples since a more random orientation of the liquid crystalline network would be expected to result in a more viscous system (cf the analogy of a polymer in both poor and good solvents). The data presented, however, do show the wealth of information that can be accrued by a systematic analysis of simple conductivity measurements.

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Ionization constants and partition coefficients of 1-arylpiperazine derivatives

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The ionization constant (pK_a) and liposolubilities (log P) of fourteen 1-arylpiperazines were determined by n-octanol/buffer partition. pK_a varied little across the entire series. Log P values ranged from less than 1 to about 2 for the highly lipophilic derivatives of the preset series. The results are discussed in relation to the extent to which these derivatives, known to be centrally active, may enter the brain.

Results have been presented recently (Caccia et al 1982; Fong et al 1982; Caccia et al 1983, 1984a) showing that centrally active drugs bearing an arylpiperazine moiety in the side-chain of their chemical structure may form 1-arylpiperazines during biotransformation in-vivo. In addition, these metabolites, known to be biochemically and pharmacologically active (Fuller et al 1978, 1981; Minard et al 1979; Rokosz-Pelc et al 1980; Maj & Lewandowska 1980; Saari et al 1983; Pettibone & Williams 1984), tend to concentrate in the brain reaching concentrations 4–74 times those in plasma, depending on which arylpiperazine derivative is given (Caccia et al 1984b). In the present study we have investigated the comparative ionization constant (K_a) and lipophilicity, as determined by n-octanol aqueous buffer partition, of fourteen 1-arylpiperazines with a view to making a preliminary assessment of the structure-brain uptake relations in 1-arylpiperazine series.

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Materials and methods

The 1-aryl-piperazines used were: 1-(2-pyrimidinyl)-piperazine (PmP) and its *p*-fluoroderivative (*p*FPmP), 1-(2-thiazolyl)-piperazine (TzP), 1-(2-pyridyl)-piperazine (PdP), 1-(2-quinolinyl)-piperazine (QuP), 1-(1,2-benzisothiazol-3-yl)-piperazine (BtP), 1-phenyl-piperazine (PP), and its *o*-methoxy (*o*OCH_3PP), *o*-methyl (*o*CH_3PP), *o*-chloro (*o*ClPP), *m*-chloro-(*m*ClPP), *p*-chloro (*p*ClPP), *m*-trifluoromethyl (*m*CF_3PP) and *p*-fluoro (*p*FPP) substituted derivatives (see Fig. 1 for chemical structures). K_a (20 °C) of 1-aryl-piperazine was determined by ultraviolet titration of 2×10^{-4} M aqueous buffer solutions of the test compound. Buffer solutions (pH 4·5–9·5) were prepared from 0·1 M potassium dihydrogen phosphate and 0·05 M sodium borate; the pH was raised by adding 0·1 M

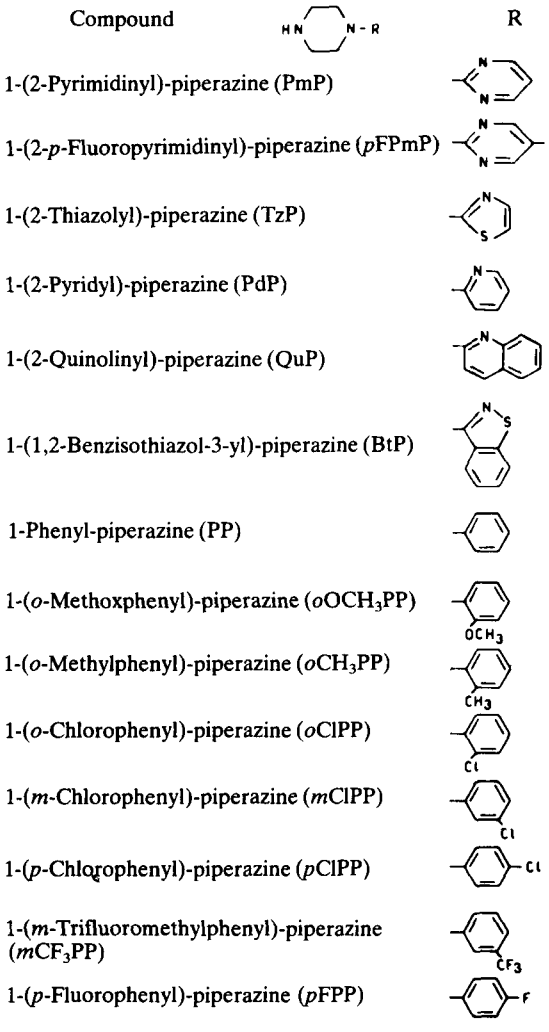

FIG. 1. Chemical structure of 1-arylpiperazine derivatives.

sodium hydroxide. The uv absortion of the aqueous layer was measured at an appropriate wavelength (250–280 nm). K_a values of 1-arylpiperazines were calculated according to the equation:

$$\varepsilon = \frac{A_H \times [H^+] \times A_B \times K_a}{[H^+] + K_a} \quad (1)$$

where A_H and A_B are, respectively, the coefficients of the ionized and unionized form and ε is the extinction coefficient at a given pH. Experimental values of ε vs pH were fitted to equation 1 by a non-linear regression iterative program. All determinations were in triplicate. In the partition studies, n-octanol and phosphate buffer solution pH 7·4–11 (prepared as above) were pre-equilibrated by shaking together, separating and storing until required. The test compound was dissolved in the buffer solution at a concentration of 2×10^{-4} M and 5 ml of the buffer solution was shaken with a suitable volume (1–5 ml) of saturated n-octanol for 1 h at 37 °C in a water bath. The mixture was then left in the water bath to separate for 30 min. A volume of 25–100 µl of the buffer phase before (C_1) and after (C_2) partition was injected into high pressure liquid chromatograph equipped with a µBondapack C_{18} reversed-phase column with solvent delivered at 1–2 ml min^{-1} (Waters Associates, Milford, MA, model 6000 pump). The mobile phase was a mixture of 0·01 M bipotassium monohydrogen phosphate-acetonitrile (70 : 30 v/v) adjusted to pH 6·5 by the addition of phosphoric acid. Compounds eluted from the column were detected at 229 and 254 nm.

The apparent partition coefficient (P_A) was obtained from the equation:

$$P_A = \frac{C_1 - C_2}{C_2} \times \frac{V_{buf}}{V_{oct}} \quad (2)$$

where V_{buf} and V_{oct} are the volumes of buffer and n-octanol used. The true partition coefficient (P) and the K_a values at 37 °C of 1-aryl-piperazines were obtained using a non-linear, least squares program on an HP85 computer:

$$P_A = \frac{P}{1 + 10^{pK_a - pH}} \quad (3)$$

The percentage ionization of the compounds at pH 7·4 and 37 °C was obtained as follows:

$$\% \text{ ionization: } \frac{1}{1 + 10^{7.4 - pK_a}} \times 100 \quad (4)$$

Results and discussion

The calculated and experimentally determined K_a, percentage ionization and partition coefficient values for each of the 1-arylpiperazines tested are listed in Table 1. The first column shows their K_a values, expressed in logarithmic form (pK_a), as determined by ultraviolet titration at room temperature (20 °C). There was little variation across the entire series the pK_a being approximately 9 (uncorrected for ionic strength) for most derivatives. TzP had the lowest pK_a (8·39) and the o-substituted-phenylpiperazines gave the highest values (9·1–9·4). The values obtained from partition data at 37 °C (see second column) were in agreement with the former pK_a values, taking into account the fall in pK_a with rising temperature. These latter values were then used to calculate the percentage ionization at pH 7·4 and 37 °C by means of equation 4. These ranged from about 80% for TzP to more than 97% for o-substituted phenylpiperazines, therefore it appears that 1-aryl-piperazines are protonated under physiological conditions.

In the fourth column are the P_A values of the compounds determined in the octanol/buffer (pH 7·4) system at 37 °C. They are the mean ± s.e.m. of three determinations. A wide range of P_A is covered (more than 90 P_A units) and therefore distinct differences in lipophilic behaviour can be expected within the series of 1-arylpiperazines. These values were then corrected for

Table 1. pK_a, percent ionization, apparent (P_A) and true (P) partition coefficient values of 1-arylpiperazine derivatives.

1-aryl-* piperazine	pK_a 20 °C	pK_a 37 °C	% Ionization pH 7·4, 37 °C	P_A (±s.e.m.) pH 7·4, 37 °C	P (log P)
PmP	8·88	8·75	95·2	0·26 ± 0·03	3·08 (0·49)
*p*FPmp	8·91	8·50	92·7	0·49 ± 0·03	9·28 (0·97)
TzP	8·39	7·94	79·7	0·78 ± 0·01	4·05 (0·61)
PdP	8·90	8·59	94·0	0·41 ± 0·02	5·51 (0·74)
QuP	8·82	8·76	95·9	10·72 ± 0·04	109·61 (2·04)
BtP	N.D.	8·68	95·0	8·87 ± 0·06	139·41 (2·14)
PP	9·02	8·29	88·6	1·19 ± 0·14	12·51 (1·10)
oOCH_3PP	9·37	8·91	97·0	0·50 ± 0·04	12·21 (1·09)
oCH_3PP	9·28	9·14	98·2	5·37 ± 0·24	136·60 (2·14)
*o*ClPP	9·13	8·94	97·2	5·66 ± 0·29	134·29 (2·13)
*m*ClPP	8·85	8·64	94·6	11·29 ± 0·37	130·68 (2·11)
*p*ClPP	8·90	8·49	92·5	8·22 ± 0·40	101·24 (2·01)
mCF_3PP	8·85	8·66	94·8	23·79 ± 0·68	270·91 (2·43)
*p*FPP	8·98	8·55	93·5	1·65 ± 0·10	19·21 (1·28)

* See Fig. 1 for key to compounds; N.D. = not measured.

ionization and expressed as the true partition coefficient (P) of the natural species (free bases). Equation 3 was used for the correction, assuming that the ionized form does not partition. These latter values with their corresponding log P values are listed in the last column. They are larger than the P_A values because all the derivatives are mostly in the ionized form at pH 7·4. PmP had the lowest P (or log P) of the series, then P values increased in the order of TzP < PdP < PP < QuP < BtP. The higher P value for PdP than for TzP despite its lower P_A is a consequence of its higher pK_a. This is also true for other derivatives such as PP and *o*OCH_3PP or *o*ClPP and *m*ClPP which had similar P values but differed about twofold in P_A values. The introduction of electron-withdrawing groups (halogen, CF_3) in the aromatic ring always increased lipophilicity. *m*CF_3PP, the highly lipophilic 1-arylpiperazine of the present series, had a P value about 20 times its unsubstituted analogue (PP). That of *o*CH_3-(electron donor) substituted phenyl-piperazine, however, was comparable to the ClPP isomers. *o*CH_3PP, ClPP isomers, *m*CF_3PP, QuP and BtP had log P values around 2, in good agreement with the mean ideal lipophilic character for brain entry and activity of centrally acting compounds (Hansch et al 1967; Timmermans et al 1977).

The blood-brain barrier acts like a lipid membrane and the lipophilicity of drugs in a series of congeners plays a major role in determining the extent to which they enter the brain (Mayer et al 1959; Brodie et al 1960). *m*CF_3PP is highly concentrated in the central nervous system and brain plasma concentration ratios even higher than 70 have been found in animal studies (Caccia et al 1985b). Brain plasma ratios of other halogenated phenylpiperazines such as *p*FPP, *m*ClPP and *o*ClPP were in the region of 15–30 in rats given psychotropic drugs containing such moieties in their side-chain (Fong et al 1982; Caccia et al 1984a); the ratio for PP and PdP is about 10 while less lipophilic 1-arylpiperazines such as TzP or PmP enter the brain to a lesser extent, achieving brain plasma ratios in the region of 4–6 after administration of either the derivatives themselves or their parent drug(s) (Caccia et al 1983, 1984a). Whether and how such differences affect these compounds' pharmacological activity cannot be established on the basis of current knowledge; pharmacological studies of 1-arylpiperazines are incomplete and fragmentary and structure-activity relations are scarce (Fuller et al 1980; Fuller & Mason 1981). A number of biochemical and pharmacological (see introduction) studies, however, indicate 1-arylpiperazines have central effects and therefore their formation after administration of drugs bearing an arylpiperazine side-chain may have pharmacological and clinical significance. This will depend on the extent of their formation as well as the degree to which they enter the brain, particularly compared to their parent drug(s). From our preliminary results it appears that 1-arylpiperazines may be classified according to their lipophilic character and that this may be related to their brain concentrations.

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A comparative study of the bioavailability of five different phenytoin preparations

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The concentration of phenytoin in saliva has been measured in 8 healthy volunteers at intervals after an intravenous dose and after single oral doses of five formulations commercially available in the United Kingdom. The six doses (all 300 mg) were given in random order and at least one week apart. There were no significant differences in the mean values of the peak saliva concentration, the time-to-peak and the area under the saliva concentration-time curve between the five oral formulations. The absolute bioavailability of phenytoin varied between 68 and 74%.

Phenytoin has been classified (Doluisio et al 1973) as a drug with 'high risk potential' with respect to bioavailability problems. The drug has a low therapeutic index and displays saturation kinetics at conventional doses (Richens 1979). Maintenance of a safe and effective steady-state plasma concentration requires that the absolute daily dose remains constant. Even minor alterations in the extent of absorption (bioavailability) can alter equilibrium substantially. It is therefore unfortunate that phenytoin has physicochemical properties which tend to render its absorption inconsistent and unreliable (Neuvonen 1979). The problems associated with differences in bioavailability between preparations of phenytoin have been documented in Europe, North America and Australasia (for references, see Neuvonen 1979). However, a comparison of the steady-state concentrations of phenytoin produced by five different preparations in a cross-over study in epileptic patients receiving regular treatment (Chen et al 1982) revealed only minor differences which, although statistically significant, were not considered to be clinically important. But claims of bioinequivalence continue to be made not only by certain manufacturers but also by epileptic patients who have experienced alteration in seizure frequency following product substitution.

We thought it reasonable to carry out a further comparison of different preparations marketed in Britain, and have examined saliva concentration-time profiles after single oral and intravenous doses in healthy volunteers.

Subjects and methods

Subjects were all healthy employees of the North Staffordshire Health Authority. All underwent physical examination and provided venous blood for determination of full blood count and biochemical indices of liver and kidney function prior to entry into the study. Written informed consent was obtained and the study received prior approval by the Ethical Committee of the Health Authority. The subjects were not permitted to take any medicines for 24 h before each study and fasted for 9 h before, and 3 h after, each dose. Each subject received 6 separate doses of phenytoin 300 mg (5 oral, 1 intravenous) in random order and at least one week apart. The oral doses were taken with 200 ml of water and the mouth was rinsed thoroughly both immediately and 15 min after the dose. The intravenous dose (Epanutin Ready-Mixed Parenteral, Parke-Davis; 300 mg in 6 ml) was given as a slow infusion in 50 ml of isotonic saline over 30 min. The oral preparations used are listed in Table 1.

Saliva samples (5 ml) for determination of phenytoin concentration were collected before and at hourly

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