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Lipid solubility of a series of drugs and its relevance to fatal poisoning

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Abstract—For 11 commonly used drugs, the n-octanol/water partition coefficient (pH 7·4 temperature 37°C) and solubility in n-octanol were determined. The drugs tested were chlorpromazine, amitriptyline, trazodone, dextropropoxyphene, diltiazem, dibucaine, amethocaine, procaine, quinidine, acetylsalicylic acid and paracetamol. For eight of the drugs, the relative lipid saturation corresponding to a fatal plasma concentration was estimated from the two parameters determined above and the median fatal blood concentrations reported in the literature. For five of those eight drugs, the estimated relative saturation in the lipid phase fell within the range 0·001–0·004 which is close to relative saturation figures in aqueous and vapour phases already published for chemicals possessing a non-specific or physical mechanism of toxicity. Since this is determined largely by their lipid solubility, it is probable that accumulation in the lipid phase is an important determinant of the lethal toxicity of drugs and chemicals with a non-specific mechanism of toxicity.

There are several measures of the hydrophobicity or lipophilicity of chemicals: these include aqueous solubility, aliphatic chain carbon numbers (e.g. for alcohols, aldehydes), reversed phase chromatography retention index or R_F values, and the partition coefficient of a substance between water and an organic solvent, membrane phospholipid or whole membrane. Currently, however, the most commonly accepted index of lipophilicity is the n-octanol/water partition coefficient; n-octanol is considered best to model the properties of biological membranes and indeed it has been shown that the free energy of transfer per methylene group for alcohols from the aqueous phase to octanol and to red cell ghost membranes is the same (Hansch & Dunn 1972; Leo et al 1971). A large compilation of partition coefficients has been published (Leo et al 1971), but in many cases the conditions of the assay were either not given or differed widely between substances and assays. Conditions of temperature and pH are particularly important and have been shown to have a major effect upon the reported partition coefficients for the β-adrenoceptor antagonists (Hellenbrecht et al 1973; Woods & Robinson 1981). For these drugs, the partition coefficient at physiological temperature (37°C) and pH (7·4) is closely correlated with their non-specific membrane activity (Hellenbrecht et al 1973; Hachisu & Koeda 1980; Hong & Turner 1982; Cassidy & Henry 1986 a, b), and a wide range of pharmacokinetic parameters, particularly central nervous system and other tissue penetration, extent of first pass metabolism, inhibition of oxidative drug metabolism and degree of plasma protein binding (Street et al 1978; Deacon et al 1981; Jack 1981; Woods and Robinson 1981; Hinderling et al 1984). The work of Woods & Robinson (1981) was the first comprehensive comparison of ten β-blocking drugs in a single study under standard conditions. The aim of the present work is to present a similarly standardized study of 11 different commonly used drugs, with an indication of the importance of the data for their toxicological properties. This has been done by calculation of the relative saturation in the lipid phase corresponding to a fatal plasma concentration, since, at equilibrium following the partitioning of a chemical between two immiscible phases, the relative saturation is the same in each phase, be it aqueous (e.g. plasma or water for aquatic species), gaseous (for volatile chemicals) or lipid (King 1985). This study aims to establish that comparison of the relative saturation data can be made between studies, provided similar conditions obtain.

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Methods

All drugs were used as supplied at a purity of 98–100%. The drugs used were chlorpromazine hydrochloride (HCl) (May & Baker Ltd), amitriptyline HCl (Parke Davis), trazodone HCl (Roussel Ltd.), dextropropoxyphene HCl (Cox Ltd), diltiazem HCl (Lorex Ltd), dibucaine HCl (Ciba Geigy Ltd), amethocaine (tetracaine) HCl (Smith and Nephew Ltd.), procaine HCl, quinidine HCl (Sigma Co.), acetylsalicylic acid (Eli Lilly) and paracetamol (Sterling Winthrop Ltd). Propranolol HCl (ICI Pharmaceuticals) was also included in the saturated n-octanol concentration determination and subsequent calculations.

Determination of n-octanol partition coefficients. The n-octanol was washed with tap water, 1 M sodium hydroxide solution and finally distilled water. The washed octanol and the phosphate-buffered saline (PBS, Dulbecco 'A') were pre-equilibrated by shaking together and then storing until required. For each drug, a solution in PBS was prepared at a concentration of 10^{-2}M, except for trazodone (10^{-3}M) and quinidine (5×10^{-3}M), and adjusted to pH 7·4; 5 mL of this solution was preincubated at 37°C before being mixed vigorously with 5 mL of pre-incubated n-octanol. (For amitriptyline, chlorpromazine and trazodone, results using 2·5 mL of n-octanol were also included to control for possible errors due to low absorbances in the aqueous phase; these results were compared with those from experiments using 5 mL octanol and little difference was found.) The mixture was allowed to separate at 37°C with gentle shaking for 30 min and then centrifuged at 700 *g* for 5 min to improve separation and eliminate emulsions. The aqueous layer was removed from under the n-octanol layer with a syringe and long needle from which air was continuously expelled from the tip, as it descended through the octanol layer. The absorbance of the aqueous layer was read in a Unicam 1800 double beam spectrophotometer, and the concentration of the drug was determined by reference to the original absorbance and to a standard curve. The weight of drug in each layer was calculated and adjusted for a lower volume of octanol (2·5 mL) if appropriate. The partition coefficient was calculated as:

(Weight of drug in n-octanol)/(Weight of drug in PBS layer)

For each drug, the assay was repeated 3–6 times and the mean value and coefficient of variation were calculated.

Saturated n-octanol concentration determinations. For each drug a saturated solution in n-octanol was prepared at 37°C and its concentration determined spectrophotometrically by reference to a standard curve, using dilutions of the saturated solution.

Calculations of relative saturation from fatal blood concentration. For seven drugs and propranolol (see Table 1) the median fatal blood concentration was obtained (Stead & Moffat 1983) and the concentration of drug in the lipid phase was determined from the product of its multiplication with the partition coefficient. The relative saturation in the lipid phase was calculated theoretically by division of this product by the concentration of the saturated solution of the drug in n-octanol.

Table 1. Partition coefficient, n-octanol solubility and relative saturation data for the eleven drugs studied and propranolol.

Drug	n-Octanol/water partition coefficient (pc) (coefficient of variation %)		Concn of saturated solution in n-octanol (S)		Median fatal blood concn (F)		Theoretical concn in lipid phase at fatal blood concn (L) ($=F\times pc$)	Theoretical relative satn in lipid phase at fatal blood concn
			g L^{-1}	mol L^{-1}	mg L^{-1}	mol L^{-1}	mol L^{-1}	(L/S)
Chlorpromazine HCl	79·7	(16·1)	438·4	1·30	5	$1{\cdot}56\times10^{-5}$	$1{\cdot}24\times10^{-3}$	0·00096
Amitriptyline HCl	74·8	(16·9)	307·6	0·98	3·3	$1{\cdot}19\times10^{-5}$	$8{\cdot}90\times10^{-4}$	0·00091
Trazodone HCl	63·3	(19·1)	20·6	0·05	—	—	—	—
Dextropropoxyphene HCl	25·9	(18·6)	184·8	0·49	15	$4{\cdot}41\times10^{-5}$	$1{\cdot}14\times10^{-3}$	0·0023
Diltiazem HCl	19·4	(9·9)	160·5	0·36	—	—	—	—
Dibucaine HCl	33·9	(25·7)	332·0	0·87	—	—	—	—
Amethocaine HCl	9·10	(21·3)	116·8	0·39	—	—	—	—
Procaine HCl	1·01	(12·5)	30·4	0·11	49	$2{\cdot}07\times10^{-4}$	$2{\cdot}09\times10^{-4}$	0·0019
Quinidine HCl	73·4	(20·4)	14·3	0·038	55	$1{\cdot}69\times10^{-4}$	$1{\cdot}28\times10^{-2}$	0·339
Acetylsalicylic acid	11·7	(25·1)	42·6	0·24	500	$2{\cdot}77\times10^{-3}$	$3{\cdot}24\times10^{-2}$	0·135
Paracetamol	1·79	(8·1)	20·0	0·13	250	$1{\cdot}65\times10^{-3}$	$2{\cdot}95\times10^{-3}$	0·0227
Propranolol HCl	20·2*	(6·6)†	54·0	0·183	9	$3{\cdot}46\times10^{-5}$	$6{\cdot}98\times10^{-4}$	0·0038

* Woods & Robinson (1981)
† Woods & Robinson, personal communication

Results

The results of the partition coefficient and saturated n-octanol concentration determinations are given in Table 1, together with calculated concentrations and relative saturation in the lipid phase.

Discussion

This study measured the apparent partition coefficient and thus relative hydrophobicity at physiological pH. The percentage of each drug in the ionized state at this pH will vary in accordance with the pK_a value of the drug and this will affect relative partitioning behaviour at this pH. Five of the drugs used (chlorpromazine, amitriptyline, dextropropoxyphene, dibucaine and amethocaine) self-associate to form micelles. However, with all but chlorpromazine, the concentration employed in the partition coefficient determination and that occurring in-vivo were lower than the critical micellar concentration (CMC) reported for each drug in saline solution (Attwood & Florence 1983) although the CMC may be further reduced by the presence of phosphate ions. For chlorpromazine, the partition coefficient may therefore have been influenced by aggregation in the aqueous phase, although partitioning of drug into octanol would continuously reduce the aqueous concentrations to below the CMC, so that the final effect upon partitioning may be minimal. Propranolol also shows significant micelle formation, but the aqueous concentration employed by Woods & Robinson (1981) was 2×10^{-4}M (Robinson, personal communication), which is well below its CMC of $9{\cdot}5\times10^{-2}$M (Attwood & Florence 1983).

The partition coefficients of dibucaine, amethocaine and procaine were of the same order as those reported by Fazly Bazaz & Salt (1983), although different conditions of temperature (22°C), pH (6·4) and aqueous medium were used in their study and may easily account for the observed small differences. Those for chlorpromazine and acetylsalicylic acid were similar to those reported in the compilation of Leo et al (1971), but the result for amitriptyline was lower than their reported value; however, the conditions of the assays were not reported in that publication.

The saturated concentration of each drug in n-octanol varied widely but showed a weak correlation with the n-octanol/water partition coefficient, i.e. the higher the partition coefficient, the higher the saturated solution concentration in n-octanol, as might be expected. However, trazodone and quinidine provided striking exceptions, with surprisingly low saturation values in n-octanol.

Calculation of the theoretical relative saturation of the lipid phase in cases of fatal poisoning was carried out for the eight drugs (see Table 1) for which adequate fatal blood concentration data existed (Stead & Moffat 1983). For chlorpromazine, amitriptyline, dextropropoxyphene, procaine and propranolol, the relative lipid saturation varied between approximately 0·001 and 0·004, a range falling close to the mean relative saturation in the aqueous phase of 0·006 (range 0·003–0·008) for fatal blood concentrations of 12 drugs reported by King (1985). The difference in these two ranges may be accounted for by deficiencies in the octanol/water model in representing partitioning into the lipid biophase (Klein 1983) and the effect of plasma protein binding. This close agreement of the relative saturations in both studies tends to support the suggestion of a non-specific hydrophobic interaction as a common mechanism of lethal toxicity for these depressant drugs. The relative saturations of toxic/narcotic concentrations of non-specific 'physical' toxins in the vapour phase ranged from 0·01 to 0·5 in a wide range of lower animals (Ferguson 1951; Rang 1960) and from 0·01 to 0·06 (one value of 0·004) in mammals (Rang 1960; Clark & Tinston 1982) and fish (Veith et al 1983); this suggests an increasing sensitivity to non-specific toxins from lower to higher organisms. The higher relative saturation obtained for quinidine is surprising since its primary mode of action is mediated by a non-specific mechanism (i.e. membrane-stabilizing activity); the drug, however, has a comparatively high reported median fatal blood concentration and a low solubility in n-octanol. Neither paracetamol nor acetylsalicylic acid are considered to be non-specifically acting toxins, both drugs having a specific action in lethal overdose.

The estimated saturation both in the gaseous phase and in the aqueous phase for many chemicals with a non-specific mode of toxicity has been shown to fall within a close range. On theoretical grounds, the saturation in the lipid phase should also fall within a close range, and this may be a more important determinant of lethal toxicity, since perturbation of membranes occurs in this phase. This study has demonstrated the close concurrence of the estimated saturations in the lipid phase at lethal blood concentrations for five depressant drugs.

For anaesthetic or hypnotic activity an octanol/water partition coefficient of 100 (log $P=2{\cdot}0$) is considered to be optimal under physiological conditions, with activity decreasing above

and below this value (Albert 1985). It is likely that for drugs and chemicals which have a non-specific membrane stabilizing action in acute overdose, this rule will also apply. It is recommended therefore that testing of new chemicals, whether pharmaceutical, industrial or agricultural, at the acute toxicity stage, should include the determination of the n-octanol/water partition coefficient under physiological conditions (pH 7·4 temperature 37°C) in addition to any determinations performed under environmental conditions for industrial or agricultural chemicals.

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References

Albert, A. (1985) Selective toxicity—the physicochemical basis of therapy. 7th Edn., London, Chapman and Hall, Chap. 15.
Attwood, D., Florence, A. (1983) Surfactant systems. 1st Edn. London, Chapman and Hall, Ch. 4.
Cassidy, S. L., Henry, J. A. (1986a) Hum. Toxicol. 5: 148–149
Cassidy, S. L., Henry, J. A. (1986b) Food Chem. Toxicol. 24: 807–809
Clark, D. G., Tinston, D. J. (1982) Hum. Toxicol. 1: 239–247
Deacon, D., Lennard, M., Bax, N., Woods, H., Tucker, G. (1981) Br. J. Clin. Pharmacol. 12: 429–431
Fazly Bazaz, B. S., Salt, W. G. (1983) Microbios 37: 45–64
Ferguson, J. (1951) Colloques Internationaux du Centre Nationale de la Recherche Scientifique, Paris, pp 25–39
Hachisu, M., Koeda, T. (1980) J. Pharmacobiodyn. 3: 183–184
Hansch, C., Dunn, W. (1972) J. Pharm. Sci. 61: 1–10
Hellenbrecht, D., Lemmer, B., Wiethold, G., Grobecker, H. (1973) Naunyn-Schmiedeberg's Arch. Pharmacol. 277: 211–226
Hinderling, P. H., Schmidlin, O., Seydel, J. (1984) J. Pharmacokinet. Biopharm. 12: 263–287
Hong, C. Y., Turner, P. (1982) Br. J. Clin. Pharmacol. 14: 269–272
Jack, D. B. (1981) Ibid. 11: 402–406
King, L. A. (1985) Hum. Toxicol. 4: 273–278
Klein, R. (1983) In: Earnshaw, J. and Steer, M. (eds) The application of laser light scattering to the study of biological motion. 1st Edn, Plenum, New York. pp 301–317
Leo, A., Hansch, C., Elkins, D. (1971) Chem. Rev. 71: 525–616
Rang, H. (1960) Br. J. Pharmacol. 15: 185–200
Stead, A., Moffat, A. (1983) Hum. Toxicol. 3: 437–464
Street, J. A., Gonda, I., Parkinson, H., Hemsworth, B. A. (1978) J. Pharm. Pharmacol. 30, (suppl): 56P
Veith, G. D., Call, D. J., Brooke, L. T. (1983) Can. J. Fish. Aquat. Sci. 40: 743–748
Woods, P. B., Robinson, M. L. (1981) J. Pharm. Pharmacol. 33: 172–173

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Inhibition of the cataleptic effect of tetrahydrocannabinol by other constituents of *Cannabis sativa* L.

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Abstract—Tetrahydrocannabinol (THC) induced catalepsy in mice, whereas a cannabis oil (6·68% w/w THC), four cannabinoids and a synthetic mixture did not. Cannabinol (CBN) and olivetol inhibited THC-induced catalepsy in the mornings and the evenings, but cannabidiol (CBD) exhibited this effect only in the evenings. A combination of CBN and CBD inhibited THC-induced catalepsy equal to that of CBN alone in the mornings, but this inhibition was greater than that produced by CBN alone in the evenings.

The psychotropic potency of cannabis is believed to be dependent upon its content of THC (Fairbairn & Pickens 1981). Cannabis has been shown to induce catalepsy in experimental animals (Paton & Pertwee 1973a) and man (Paton & Pertwee 1973b), and this response has been presumed to correlate with psychotic effects. Cannabis oil contains a mixture of cannabinoids and other substances and is generally more potent than the herb or the resin. Whilst THC produces catalepsy in mice, at least one other cannabinoid, CBD, is inactive (Pertwee 1972).

Previous reports have postulated a link between prostaglandin (PG) levels and the central effects of cannabis (Fairbairn & Pickens 1979), however we have shown a close similarity in the effects of cannabinoids on PG release from cell culture (Barrett et al 1985) and cell free assays for enzymes of arachidonate metabolism (Evans et al 1987a). Clearly if THC is unique in possessing central activity, PG metabolism is an unlikely target. It was therefore important to re-examine the cataleptic response to cannabis, including an evaluation of the natural oil, synthetic mixtures of cannabinoids, and the pure cannabinoids.

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Materials and methods

Drug preparation. Stock solutions were prepared (10 mg mL^{-1}) in redistilled ethanol and stored at −4°C. Serial dilutions were made with 0·9% w/v sodium chloride containing 2·5% w/v Tween 80. The final concentrations of ethanol did not exceed 1% v/v and this concentration had no effect in the cateleptic tests performed.

Animals. Male albino CD1 mice (Charles River), 22–30 g, were maintained at 30–32°C.

Cannabinoids (THC, Δ^1-tetrahydrocannabinol; CBN, cannabinol; CBD, cannabidiol; CBG, cannabigerol; olivetol) were purchased from Makor Chemicals Ltd, Israel. Cannabis (strain UNC 335) was cultivated and an oil produced as previously described (Barrett et al 1985).

Drugs were administered (1 mL/100 g weight) by gavage; controls received only the vehicle. Two hours later, animals were gently placed on a wire ring (Pertwee 1972) and the sum of the time in seconds during which the animal remained immobile over a 5 min period was recorded. Catelepsy was expressed as the