Liquid Membrane Phenomena in Haloperidol Action

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Abstract □ Haloperidol, a surface-active neuroleptic drug, was shown to generate a liquid membrane on a supporting membrane. Transport of adrenalin, noradrenalin, dopamine, serotonin, histamine, glutamic acid, γ-aminobutyric acid, and sodium, potassium, and calcium ions through the haloperidol liquid membrane was studied. The data indicate that the phenomenon of a liquid membrane plays a significant role in the mechanism of action of haloperidol.

Keyphrases □ Liquid membrane phenomena—effect on haloperidol action □ Haloperidol—liquid membrane phenomena, effect on mechanism of action □ Surface-active drugs—haloperidol, effect of liquid membrane phenomena

A wide variety of biologically active agents have been shown to be surface active (1–4). This does not appear to be a coincidence. In a number of cases a definite correlation between surface activity and biological effects has been demonstrated (1, 3, 4). Most of the potent neuroleptics are known to behave like powerful surface-active agents (2) and the surface activities of neuroleptic drugs also correlate with their clinical potencies (1). Palm *et al.* (5) concluded that regardless of the chemical structure, mainly the surface activity of psychotropic drugs determines their potencies toward all kinds of membranes, especially that of catecholamine-storing particles. Surface-active agents have been shown (6–8) to generate liquid membranes which completely cover the interface at or above the critical micelle concentration of the surfactants when added to water or aqueous solutions. Therefore, it is likely that the liquid membrane phenomena may have an important role in the mechanism of action of such drugs, and deserves a systematic investigation. Haloperidol is a neuroleptic drug belonging to the class of butyrophenones and is known to be surface active in nature (2).

Haloperidol acts by modifying the permeabilities of catecholamines and a few neurotransmitter amino acids in biological cells (2). To date, this effect has been explained solely on the basis of specific drug interaction with the receptors. No serious attempt has been made to assess the contribution of the liquid membrane to the mechanism of its action. Experiments were designed to demonstrate the existence of a haloperidol liquid membrane at the interface. Data on the transport of endogenous amines, neurotransmitter amino acids, and sodium, potassium, and calcium ions through the haloperidol liquid membrane were obtained. A cellulose acetate membrane–water interface was chosen as a site for the formation of the liquid membrane. In this way, any active and specific interaction of the drug with the membrane that alters the permeabilities of relevant permeants was ruled out and only data on passive transport was obtained.

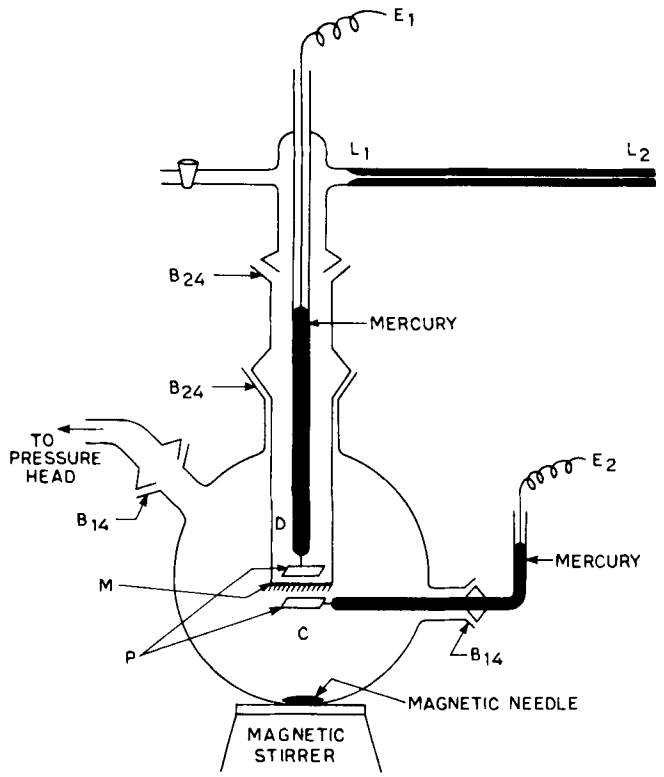

Figure 1—*The transport cell. Key: M, supporting membrane (cellulose acetate millipore filter); P, bright platinum electrodes; L_1L_2, 17-cm capillary tube with 1.33 × 10^{-1} cm diameter and E_1 and E_2, electrode terminals. Volume of Compartments C and D are 590 and 50 ml, respectively.*

EXPERIMENTAL

Materials—Haloperidol[1], dopamine chlorhydrate[2], adrenalin hydrogen tartrate[2], L-noradrenalin[3], serotonin creatinine sulfate[4], histamine acid phosphate[5], L-glutamic acid[5], γ-aminobutyric acid[5] (I), sodium chloride[6], potassium chloride[6], calcium chloride[6], and water glass-distilled over potassium permanganate were used.

Methods—The critical micelle concentration (CMC) of aqueous haloperidol was determined from the plots of surface tension *versus* concentration at 37 ± 0.1°. The surface tensions were measured using the method of capillary rise. The CMC value found for aqueous solutions of haloperidol, was 1.064 × 10^{-6} *M*. To prepare the aqueous solutions of haloperidol, the weight of drug necessary to attain the desired concentration was dissolved in ethanol and added with constant stirring to the aqueous phase. The stirring was continued for ~12 hr. In the aqueous solutions of haloperidol thus prepared, the final concentration of ethanol was never allowed to exceed 0.1% (v/v) because a control experiment showed that 0.1% (v/v) solution of ethanol in water did not lower the surface tension of water to any measurable extent.

The all-glass cell described earlier (8) was used for the transport studies (Fig. 1). The transport cell was separated into two compartments by a cellulose acetate millipore filter[7] which acted as a support for the liquid membrane.

For measurements of hydraulic permeability, aqueous haloperidol solutions of various concentrations ranging from 0 to 1.064 × 10^{-5} *M* were filled in Compartment C of the transport cell (Fig. 1), while Compartment D was filled with distilled water. The concentration range was selected

[1] B. P. Searle (India).
[2] Loba Chemie.
[3] Fluka A.G..
[4] Koch Light Laboratories Ltd.
[5] BDH.
[6] Analar grade.
[7] Sartorius, Cat. No. 11107 of thickness 1 × 10^{-4} m and area 5.373 × 10^{-5} m^2.

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to get data on both the lower and higher sides of the CMC of haloperidol. Known pressures were applied on Compartment C by adjusting the pressure head and the resulting volume flux was measured by noting the rate of advancement of the liquid meniscus in capillary L_1L_2 with a cathetometer reading up to 0.001 cm and a stopwatch reading up to 0.1 sec. The magnitude of the applied pressures also was measured by noting the position of the pressure head, with a cathetometer reading up to 0.001 cm. During the volume flux measurements the solution in Compartment C was well stirred and electrodes E_1 and E_2 were short circuited so that the electro-osmotic back flow that developed due to streaming potentials did not interfere with the observations.

To measure permeability of endogenous amines, amino acids, and sodium, potassium, and calcium ions, two sets of experiments were performed. In the first set of experiments, Compartment C of the transport cell was filled with the respective permeants, prepared in 4.256×10^{-6} *M* aqueous solution of haloperidol and Compartment D was filled with distilled water. In the second set of experiments Compartment D was filled with 4.256×10^{-6} *M* solution of haloperidol and Compartment C was filled with aqueous solutions of the permeants. No haloperidol was used in the control experiments. The initial concentrations chosen for the endogenous amines, amino acids, and cations were comparable to their concentrations in the vicinity of nervous tissue. The condition of no net volume flux ($J_v = 0$) was attained by adjusting the pressure head attached to Compartment C of the transport cell so that the liquid meniscus in capillary L_1L_2 remained stationary. After a known period of time, the concentration of the permeant in the other compartment was measured. The amount of permeant gained by Compartment D divided by the time and the area of the membrane gave the value of solute flux (J_s). The value of solute permeability was estimated using the definition (9, 10):

$$\left(\frac{J_s}{\Delta\pi}\right)_{Ju=0} = \omega \qquad \text{(Eq. 1)}$$

where $\Delta\pi$ is the osmotic pressure difference. The value of $\Delta\pi$ used in the calculation of ω was the average of the $\Delta\pi$ values at the beginning ($t = 0$) and end of the experiment. During the permeability measurements, the solution in Compartment C was kept well stirred. All measurements were made at constant temperature by placing the transport cell (Fig. 1) in a thermostat set at 37 ± 0.1°.

Estimations—The amounts of the various permeants transported to Compartment D were estimated as follows:

Endogenous Amines—The amounts of the catecholamines, namely dopamine, noradrenalin, and adrenalin, were estimated fluorometrically at 325 nm (11). Though the fluorescence maximum of serotonin is reported to be 330 nm (12), it was measured at 325 nm since a quartz cell was used. Histamine was estimated by measuring the fluorophor derived from its reaction with *o*-phthalaldehyde[8] (11, 13) at 450 nm. A UV-visible–near IR spectrophotometer[9] with fluorescence attachment was used for fluorescence measurements.

Amino Acids—The amounts of glutamic acid and γ-aminobutyric acid were estimated by spectrophotometric[10] determination of their reaction products with ninhydrin[5] (14) at 570 nm.

Cations—The amounts of sodium, potassium, and calcium ions were determined using an atomic absorption spectrophotometer[11] using emission mode.

The estimations were carried out using the following lines: sodium, 589.6 nm; potassium, 766.5 nm; and calcium, 422.7 nm.

RESULTS AND DISCUSSION

Liquid Membrane Formation—From the hydraulic permeability data at various haloperidol concentrations (Fig. 2), it is obvious that the linear relationship is obeyed in all cases:

$$J_v = L\,\Delta P \qquad \text{(Eq. 2)}$$

where J_v represents the volume flux per unit area of the membrane, ΔP is the applied pressure difference, and L is the hydraulic conductivity coefficient. The values of L show (Table I) a progressive decrease as the haloperidol concentration is increased from 0 to its CMC value, *i.e.*, 1.064×10^{-6} *M*. When the haloperidol concentration is increased further, the value of L also decreases, but much less than the decrease observed up to the CMC value of haloperidol. This trend is in keeping with Kesting's

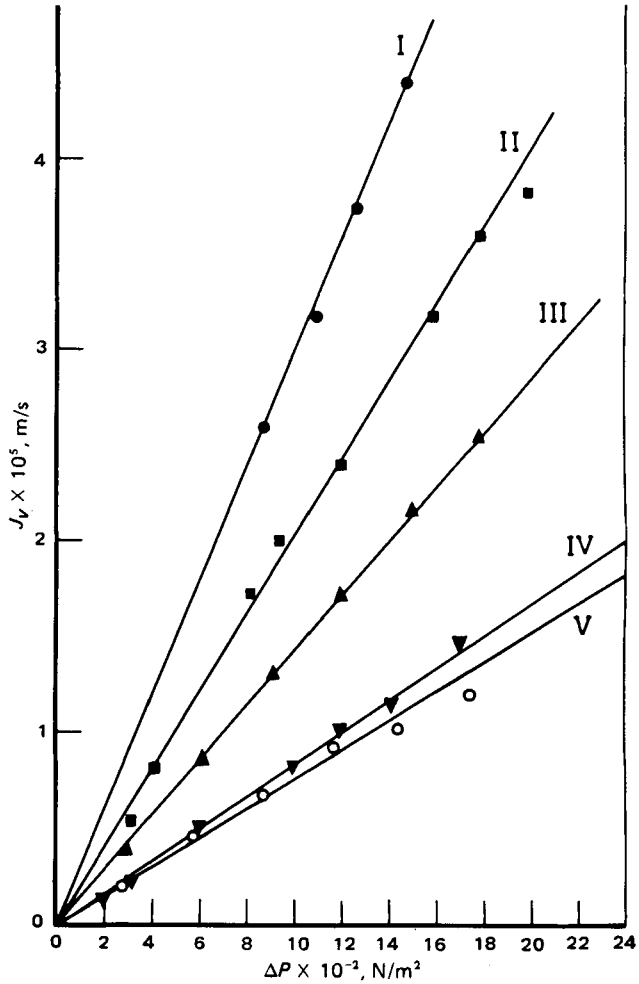


Figure 2—*Hydraulic permeability data. Curves I, II, III, IV, and V are for 0, 1.064×10^{-7}, 5.32×10^{-7}, 1.064×10^{-6}, and 1.064×10^{-5} M haloperidol concentrations, respectively.*

liquid membrane hypothesis (6–8), where the surfactant concentration is increased, the supporting membrane gets progressively covered with the surfactant layer liquid membrane. Then, at the CMC, the supporting membrane is completely covered with the liquid membrane. The decrease in the value of L beyond the CMC of haloperidol, could possibly be due to densing of the liquid membrane (6).

In light of the mosaic membrane model (15–17) analysis of the flow data (Fig. 2, Table I) supports the existence of the liquid membrane in series with the supporting membrane. At concentrations lower than the CMC, the supporting membrane is only partially covered with the liquid membrane. The equation for the volume flux of water for such a case can be written as:

$$J_v = \left[L^c\left(\frac{A^c}{A^c + A^s}\right) + L^s\left(\frac{A^s}{A^c + A^s}\right)\right]\Delta P \qquad \text{(Eq. 3)}$$

where A represents area of the membrane denoted by the superscripts, and superscripts c and s represent the bare supporting membrane and the supporting membrane covered with the liquid membrane, respectively. In the present case L^c represents the value of the hydraulic conductivity when no haloperidol was used, and L^s represents the value of the hydraulic conductivity when the haloperidol concentration equals its CMC. At half the CMC, the fraction of the total area covered with the liquid membrane will be halved and, therefore, the value of L should be equal to $(L^c + L^s/2)$. Similarly, for 0.1 CMC of haloperidol, the value of L should equal $(0.9\,L^c + 0.1\,L^s)$. The values of L were computed in this way, corresponding to the two haloperidol concentrations given in Table I. These values match the experimentally determined values well.

Solute Permeability in the Presence of Haloperidol—The value of ω for endogenous amines, amino acids, and cations are given in Table II. To make sure that the supporting membrane of the transport cell was completely covered with the haloperidol liquid membrane, the concentration of haloperidol chosen for the present study was 4.256×10^{-6} *M*, which is much higher than its critical micelle concentration. Haloperidol,

[8] Sigma Chemical Co.
[9] Varian Cary 17-D spectrophotometer.
[10] Bausch and Lomb Spectronic-20.
[11] Perkin Elmer, model 306.

Table I—Values of L at Various Haloperidol Concentrations

	Haloperidol Concentration × 10^7, M				
	0	1.064 (0.1 CMC)	5.320 (0.5 CMC)	10.64 (CMC)	106.4
$L^a \times 10^8$ (m^3/s N)	2.804 ± 0.4368	2.095 ± 0.1273	1.603 ± 0.2015	0.7993 ± 0.0692	0.7662 ± 0.0216
$L^b \times 10^8$ (m^3/s N)	—	2.6035 ± 0.3996	1.8017 ± 0.2510	—	—

[a] Experimental values. [b] Calculated values on the basis of mosaic model (Eq. 3).

being surface active, has both hydrophobic and hydrophilic parts in its structure. The orientation of its molecules will, therefore, be significant when it forms a liquid membrane. The hydrophobic ends of the haloperidol molecules would be preferentially oriented toward the hydrophobic supporting membrane and their hydrophilic ends will face outwards, away from the supporting membrane. When haloperidol is in compartment C of the transport cell (first set of experiments) the haloperidol liquid membrane will present a polar surface to the permeant present in the same compartment. In the second set of experiments, however, where haloperidol is in Compartment D of the transport cell and the aqueous solution of the permeant is in Compartment C, the haloperidol liquid membrane would present a hydrophobic surface to the permeant. Therefore, the orientation of haloperidol molecules with respect to approaching permeant would be different in the two sets of experiments.

The values of ω given in Table II indicate that when the hydrophobic surface of the haloperidol liquid membrane faces the approaching permeant (second experiment), a marked decrease in their permeability is observed. The haloperidol liquid membrane, thus, offers resistance to the transport of these permeants in this specific orientation. This reduction in the passive transport of biogenic amines, amino acids, and cations is likely to be accompanied by a reduction in their active transport. This occurs because the access of these permeants to the active carrier site of the biological membrane is likely to be effectively reduced due to the resistance of the haloperidol liquid membrane. The results also indicate that this specific orientation of haloperidol molecules with hydrophobic ends facing the catecholamines and amino acids, would be necessary for the liquid membrane to resist the flow of these species. In the first set of experiments, where haloperidol orients its hydrophilic ends toward the catecholamines or amino acids, the permeability of these substances is increased in the presence of haloperidol. This indicates that the orientation of haloperidol with its hydrophobic ends facing the permeants would be necessary even in biological cells.

In cells, haloperidol reduces the permeability of catecholamines (2). Despite the fact that the present experiments were carried out using a cellulose acetate membrane, the results are similar to those observed in biological cells. This indicates that the liquid membrane generated by haloperidol contributes to the resistance of the flow of catecholamines.

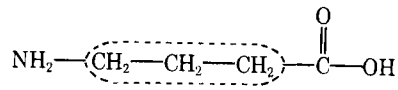
The haloperidol liquid membrane also resists the flow of glutamic acid and γ-aminobutyric acid (Table II). The antiemetic and antipsychotic actions of haloperidol are explained on the basis of reduced permeability of dopamine to biological cells, which is under the influence of the γ-aminobutyric acid–glutamic acid system (2). To investigate whether a similar trend is observed on nonspecific membranes, the dopamine permeability was measured in the presence of γ-aminobutyric acid. Interestingly, the present set of experiments also indicates that the presence

Table II—Solute Permeability ω of Endogenous Amines, Amino Acids, and Cations in Presence of 4.256×10^{-6} M Haloperidol.

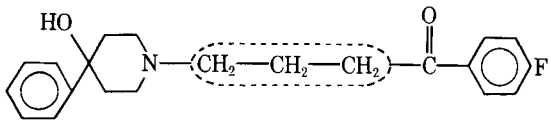
	ω_1[a] × 10^{12} moles/s N	ω_2[b] × 10^{12} moles/s N	ω_3[c] × 10^{12} moles/s N	ω_4[d] × 10^{12} moles/s N
Dopamine	887.3	680.0	2607.0	274.4
Noradrenalin	75.8	65.9	294.3	
Adrenalin	50.7	undetectable	237.4	
Serotonin	193.7	94.5	348.1	
Histamine	48.8	109.1	318.8	
Glutamic acid	58.9	47.3	81.0	
γ-Aminobutyric acid	119.8	86.6	152.2	
Sodium (chloride)	172.9	53.4	70.7	
Potassium (chloride)	175.5	157.1	101.3	
Calcium (chloride)	119.2	111.7	106.8	

[a] ω_1: control value—when no haloperidol was used. [b] ω_2: haloperidol in Compartment D of the transport cell. [c] ω_3: haloperidol in Compartment C of the transport cell. [d] ω_4: in the presence of γ-aminobutyric acid and haloperidol.

hydrophobic



γ-aminobutyric acid



haloperidol

of γ-aminobutyric acid increases resistance to the transport of dopamine through haloperidol liquid membrane (Table II). This can be explained by a strengthening of the hydrophobic core of haloperidol liquid membrane by γ-aminobutyric acid, which is obvious from the similarity of the hydrophobic components of their structures.

It is tempting to suggest that increased passive resistance to the flow of dopamine in the presence of γ-aminobutyric acid, coupled with the resistance to the flow of glutamic acid by the haloperidol liquid membrane, is likely to contribute to the mechanism of action of haloperidol.

It was reported (18) that haloperidol is considerably more potent on a milligram basis than chlorpromazine *in vivo*. The formation of liquid membrane phenomena might explain this. Because haloperidol exhibits greater surface activity than chlorpromazine (1, 2), the former will form liquid membranes at a lesser concentration, making it pharmacologically effective even at a comparatively lower concentration.

The present study shows that haloperidol reduces the permeability of serotonin (Table II). This agrees with the observations reported on biological cells (19). The extrapyramidal effects of antipsychotic drugs like haloperidol reportedly are resistant to levodopa therapy (20). Since reduced concentration of serotonin in cerebrospinal fluid has also been linked with a defect of extrapyramidal function (21, 22), the reduced permeability of serotonin in the presence of antipsychotic drugs like haloperidol offers a clue to the causation of extrapyramidal symptoms.

The observation of increased permeability of histamine in the presence of haloperidol, and its biological implication, if any, remains to be explained.

The resistance offered by haloperidol liquid membrane to the flow of sodium, potassium, and calcium cations is probably due to hydrophilicity of the ions. Unlike the observation in the case of endogeneous amines and amino acids, even when the hydrophilic ends of haloperidol are facing the approaching cations, the permeability of these ions is reduced (Table II). This may be because the ions are more hydrophilic and experience more resistance to their flow. This observation may have some biological implications relative to nerve conduction.

REFERENCES

(1) P. Seeman and H. S. Bialy, *Biochem. Pharmacol.*, **12**, 1181 (1963).

(2) P. A. J. Janssen, *Int. J. Neuropsychiatry*, **S**, 10 (1967).

(3) P. Seeman, *Pharmacol. Rev.*, **24**, 583 (1972).

(4) A. Felmeister, *J. Pharm. Sci.*, **61**, 151 (1972).

(5) D. Palm, H. Grobecker, and I. J. Bak, in "Bayer Symposium—II. New Aspects of Storage and Release Mechanisms of Catecholamines," H. J. Shumann and G. Kroneberg, Eds., Springer-Verlag, Berlin, 1970, pp 188–198.

(6) R. E. Kesting, W. J. Subcasky, and J. D. Paton, *J. Colloid Interface Sci.*, **28**, 156 (1968).

(7) R. C. Srivastava and S. Yadav, *ibid.*, **69**, 280 (1979).
(8) R. C. Srivastava and R. P. S. Jakhar, *J. Phys. Chem.*, **85**, 1457 (1981).
(9) A. Katchalsky and P. F. Curran, "Nonequilibrium Thermodynamics in Biophysics," Harvard University Press, Cambridge, Mass., 1967, pp 113–116.
(10) A. Katchalsky and O. Kedem, *Biophys. J.*, **2**, 53 (1962).
(11) S. Udenfriend, "Fluorescence Assay in Biology and Medicine," vol. I, Academic, New York, N.Y., 1969, p. 140.
(12) R. L. Bowman, P. A. Caulfield, and S. Udenfriend, *Science*, **122**, 32 (1955).
(13) D. von Redlich and D. Glick, *Anal. Biochem.*, **10**, 459 (1965).
(14) S. Moore and W. H. Stein, *J. Biol. Chem.*, **21**, 907 (1954).
(15) K. S. Speigler and O. Kedem, *Desalination*, **1**, 311 (1966).
(16) T. K. Sherwood, P. L. T. Brain, and R. E. Fischer, *Ind. Eng., Chem. Fundam.*, **6**, 2 (1967).
(17) F. L. Harris, G. B. Humphreys, and K. S. Spiegler in "Membrane Separation Processes," Elsevier, Amsterdam, The Netherlands, 1966, pp 126.
(18) S. H. Snyder, *Am. J. Psychiatry*, **133**, 197 (1976).
(19) M. Grabowska, *Pol. J. Pharmacol.*, **28**, 253 (1976).
(20) R. Byck, in "Pharmacological Basis of Therapeutics," 5th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1975, p. 172.
(21) O. Hornykiewicz, *Pharmacol. Rev.*, **18**, 925 (1966).
(22) T. N. Chase and D. L. Murphy, *Ann. Rev. Pharmacol.*, **13**, 188 (1973).

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High-Pressure Liquid Chromatographic Determination of Promethazine Plasma Levels in the Dog After Oral, Intramuscular, and Intravenous Dosage

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Abstract □ Plasma levels of promethazine were determined using a high-pressure liquid chromatographic procedure incorporating a fixed wavelength (254 nm) UV detector, following single 50-mg intravenous, intramuscular, and oral doses to two male dogs. Initial plasma promethazine concentrations following intravenous doses were 556 and 535 ng/ml in the two dogs. The subsequent decline in drug levels were satisfactorily described by a triexponential function. Peak promethazine levels of 76 and 64 ng/ml were obtained 0.5 hr following intramuscular doses. Peak levels for the oral doses were 10.6 and 11.0 ng/ml occurring 2 hr after dosing. The apparent biological half-life of promethazine, obtained from only 2–3 data points, varied from 8.5 to 27.7 hr. Areas under the promethazine plasma curves, compared to values obtained from intravenous doses between 0 and 24 hr, indicated that systemic availability of intact drug was 55–73% following intramuscular injection and 8.3–9.5% following oral administration.

Keyphrases □ High-pressure liquid chromatography—determination of promethazine plasma levels after oral, intramuscular, and intravenous dosage, dogs □ Promethazine—high-pressure liquid chromatographic determination of plasma levels after oral, intramuscular, and intravenous dosage, dogs □ Pharmacokinetics—high-pressure liquid chromatographic determination of promethazine plasma levels after oral, intramuscular, and intravenous dosage, dogs

Promethazine has been used extensively for the control of allergy and as a sedative and antiemetic; however, little information is available on its bioavailability from oral dosage forms or on its pharmacokinetics. This has been due to the absence of suitable methods for determining promethazine in body fluids.

Gas chromatographic procedures have been used to measure promethazine (1, 2) and other phenothiazines (3, 4), but these methods lack sensitivity and require extensive sample preparation before chromatography. Studies in this laboratory showed that unless promethazine is separated from its various metabolites prior to gas chromatography, spurious results may result from on-column reduction of oxidized metabolites to the parent compound (5).

High-pressure liquid chromatographic (HPLC) procedures for promethazine have been reported recently (6–8). Two of the procedures (6, 7) require either large samples or prior derivatization, while the other (8) requires the use of electrochemical detection. The lack of assays suitable for routine use is the major cause for the scarcity of information on the pharmacokinetics of promethazine in animals (2, 9) and humans (1, 10) in the literature.

The use of a specific and sensitive HPLC assay to measure plasma promethazine levels following single oral, intramuscular, and intravenous doses to male dogs is described. Preliminary details of the liquid chromatographic procedure and comparisons with a gas chromatographic assay have been described elsewhere (5).

EXPERIMENTAL

Assay for Promethazine in Plasma—To 2 ml of plasma were added 200 μl of a 0.56-μg/ml aqueous solution of chlorpromazine hydrochloride (equivalent to 0.5 μg/ml of chlorpromazine) as internal standard, 0.5 ml of 1.0 *N* sodium hydroxide, and 8 ml of methylene chloride. After shaking for 15 min at low speed on a horizontal shaker and centrifuging at 3000×*g* for 5 min, the upper aqueous phase was discarded by aspiration. The organic phase was transferred to a clean tube and evaporated to dryness under nitrogen at room temperature. The tube was rinsed with 1 ml of methylene chloride and was again evaporated to dryness. The residue was reconstituted in 60 μl of the chromatographic mobile phase by vortexing, centrifuged at 3000×*g* for 1 min, and 20 μl of the supernate was injected into the chromatograph.

The HPLC consisted of a solvent pump[1]; a fixed-volume (20 μl) injection valve[2]; a 10-μm particle size, reversed-phase octadecyl column[3]

[1] Model 110, Altex Scientific, Berkeley, Calif.
[2] Rheodyne sample injector.
[3] Lichrosorb C-18 reversed-phase column, Altex Scientific, Berkeley, Calif.

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