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HISTAMINE, THEOPHYLLINE AND TRYPTAMINE TRANSPORT THROUGH LIPID BILAYER MEMBRANES

JOHN GUTKNECHT and ANNE WALTER

Department of Physiology, Duke University, Marine Laboratory, Beaufort, NC 28516 (U.S.A.)

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Diffusion of histamine, theophylline and tryptamine through planar lipid bilayer membranes was studied as a function of pH. Membranes were made of egg phosphatidylcholine plus cholesterol (1 : 1 mol ratio) in tetradecane. Tracer fluxes and electrical conductances were used to estimate the permeabilities to nonionic and ionic species. Only the nonionic forms crossed the membrane at a significant rate. The membrane permeabilities to the nonionic species were: histamine, $3.5 \cdot 10^{-5}$ cm $\cdot s^{-1}$; theophylline, $2.9 \cdot 10^{-4}$ cm $\cdot s^{-1}$; and tryptamine, $1.8 \cdot 10^{-1}$ cm $\cdot s^{-1}$. Chemical reactions in the unstirred layers are important in the transport of tryptamine and theophylline, but not histamine. For example, as pH decreased from 10.0 to 7.5 the ratio of nonionic (B) to ionic (BH^+) tryptamine decreased by 300-fold, but the total tryptamine permeability decreased only 3-fold. The relative insensitivity of the total tryptamine permeability to the ratio, $[B]/[BH^+]$, is due to the rapid interconversion of B and BH^+ in the instirred layers. Our model describing diffusion and reaction in the unstirred layers can explain some 'anomalous' relationships between pH and weak acid/base transport through lipid bilayer and biological membranes.

Introduction

Transport of weak acids and weak bases is important in all biological membranes. Generally, the nonionic forms of weak acids and bases are much more permeant than the ionic forms. However, chemical reactions between acid/base, H^+/OH^- and buffers at the membrane surface often play an important role in controlling the rate of transport through the membrane. These chemical reactions in unstirred layers can cause transport kinetics which deviate from the predictions of simple nonionic diffusion. Chemical reactions in unstirred layers may play an important role in the transport of any solute which exists in two or more chemical forms, e.g., halogens and halides, weak acids, weak bases and heavy metals [1–5].

In this study we measured the permeabilities of lipid bilayer membranes to several compounds which are important in physiology and pharmacology, i.e., histamine, theophylline and tryptamine. We found that only the nonionic forms of these compounds cross the membrane at a significant rate. Under physiological conditions, chemical reactions in the unstirred layers are especially important in controlling tryptamine transport. Our model can explain some 'anomalous' transport kinetics observed previously for weak acid/base transport through lipid bilayers and biological membranes.

Theory

A membrane and associated unstirred layers are analogous to conductances in series. The equation relating the one-way flux of a permeant weak base (J_B) to the concentrations of the nonionic (B) and

Abbreviations. Caps, 3-(cyclohexylamino)-1-propanesulfonic acid; Ches, 2-(*N*-cyclohexylamino)ethanesulfonic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, Tricine, *N*-tris(hydroxymethyl)methylglycine.

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ionic (HB^+) forms is.

$$\frac{1}{J_B} = \frac{1}{P^{ul}([B] + [HB^+])} + \frac{1}{P_B^m[B]} \quad (1)$$

where P^{ul} is the unstirred layer permeability and P_B^m is the membrane permeability to B. This model assumes that (1) only B crosses the membrane at a significant rate, (2) chemical reactions between B, HB^+, H^+, OH^- and buffer are at equilibrium throughout the unstirred layer, (3) the unstirred layer permeabilities to B and HB^+ are similar and (4) aqueous solutions are symmetrical or sufficiently buffered so that no pH gradients exist in the unstirred layers.

Eqn. 1 can be rearranged to give

$$\frac{1}{P^t} = \frac{[B^t]}{[B]\,P_B^m} + \frac{1}{P^{ul}} \quad (2)$$

where $[B^t]$ is the total weak base concentration, i.e., $[B] + [HB^+]$, and P^t is the total permeability coefficient, i.e., $J_B/[B^t]$. If the assumptions listed above are correct, a plot of $1/P^t$ vs. $[B^t]/[B]$ will yield a straight line with a slope of $1/P_B^m$ and an intercept of $1/P^{ul}$. Thus, Eqn. 2 provides a convenient way to measure both the membrane and unstirred layer permeabilities. A similar equation can be derived to estimate the membrane and unstirred layer permeabilities to a weak acid, i.e., HA and A^- [2–4].

Materials and Methods

Lipid bilayer membranes were made by the brush technique of Mueller and Rudin [6]. Membranes were formed from a mixture of egg phosphatidylcholine (30 mg · ml^{-1}) plus cholesterol (15 mg · ml^{-1}) (1 1 mol ratio) in tetradecane. Tetradecane was used because capacitance measurements have shown that phosphatidylcholine-cholesterol-tetradecane bilayers contain very little hydrocarbon solvent [7]. Membranes were formed on a 1.8 mm^2 hole in a polyethylene partition which separated two magnetically stirred solutions of 1.1 ml each.

The aqueous solutions contained NaCl (90 mM), pH buffer (10 mM) and various test solutes at concentrations ranging from 1–100 μM. In order to vary the ratio of $[HA]$ to $[A^-]$ (or $[B]$ to $[HB^+]$) we varied the pH as described by the Henderson-Hasselbalch equation. Experiments were conducted over a pH range of 7.5 to 10.6, and solutions were buffered with either HCO_3^-/CO_3^{2-}, Ches, Caps, Tris, Tricine or Hepes. The temperature was 24 ± 2°C.

After a stable membrane was formed, 5–15 μCi of ^{3}H-labeled solute were injected into the rear compartment. The rate of appearance of radioactivity in the front compartment was measured by continuous perfusion (1–2 ml · min^{-1}) and collection of samples at 3-min intervals. The samples were collected by aspiration into a vacuum trap. During the flux experiment the rear compartment was sampled with a microsyringe. Radioactivity was measured in a liquid scintillation counter.

The one-way flux of solute was calculated by the equation.

$$J = \frac{^3H^F}{tA(SA^R)} \quad (3)$$

where J is the flux (mol · cm^{-2} · s^{-1}), $^3H^F$ is the total amount of tracer (cpm) entering the front compartment during the time interval t (s), A is the surface area of the membrane (cm^2) and SA^R is the specific activity of tracer in the rear compartment (cpm · mol^{-1}). In most experiments the test solute was present only in the rear (*cis*) solution, so the one-way flux was equal to the net flux.

We measured the membrane resistance at 3-min intervals by applying a known voltage pulse across the membrane in series with a known resistance (voltage divider circuit). The membrane potential was recorded as the potential difference between two calomel-KCl electrodes which made contact with the front and rear solutions.

[^{3}H]Histamine, [^{3}H]theophylline and [^{3}H]tryptamine were obtained from Amersham (Arlington Heights, IL). Egg phosphatidylcholine was obtained from Lipid Products (Surrey, U.K.). Cholesterol, *n*-tetradecane and pH buffers were obtained from Sigma Chemical Co. (St. Louis, MO)

Results

Histamine fluxes were measured at pH 9 8 and 10.6. The total permeabilities were (1.82 ± 0.31) · 10^{-5} cm · s^{-1} (pH 9.8) and (2.82 ± 0.06) · 10^{-5} cm · s^{-1} (pH 10.6). Assuming that only nonionic histamine crosses the membrane and that the $pK = 9.8$ [8], we

calculated the permeability to nonionic histamine to be $(3.64 \pm 0.62) \cdot 10^{-5}$ cm $\cdot$ s^{-1} at pH 9.8 and $(3.28 \pm 0.56) \cdot 10^{-5}$ cm $\cdot$ s^{-1} at pH 10.6. The similarity between the calculated nonionic permeabilities at pH 9.8 and 10.6 indicates that only nonionic histamine is crossing the membrane. This conclusion was substantiated by the observation that a membrane voltage of 60 mV had no effect on the one-way flux of histamine (Fig. 1). Histamine at concentrations up to 100 μM had no effect on membrane conductance, which ranged from 50 to 300 nS $\cdot$ cm^{-2}. Since the total observed permeability is less than 10% of the unstirred layer permeability (see below), no unstirred layer correction was applied to the data, which were pooled to give a single permeability coefficient of $(3.46 \pm 0.42) \cdot 10^{-5}$ cm $\cdot$ s^{-1} (Table I).

Theophylline fluxes were measured over a pH range of 7.7 to 9.4 at theophylline concentrations ranging from 1 to 3 μM. The data, plotted according to Eqn. 2. are shown in Fig. 2. Linear regression analysis yielded a membrane permeability to nonionic theophylline of $2.92 \cdot 10^{-4}$ cm $\cdot$ s^{-1} (Table I). The linearity of the relation between $1/P^t$ and $[A^t]/[HA]$ indicates that only HA is crossing the membrane at a significant rate. The unstirred layer permeability (reciprocal of the intercept) is about $4.5 \cdot 10^{-4}$ cm $\cdot$ s^{-1}. The unstirred layer thickness is defined operationally as D/P^{ul}, where D is the diffusion coefficient in water. Assuming that D is $6 \cdot 10^{-6}$ cm^2 $\cdot$ s^{-1}, the unstirred layer thickness is about 130 μM, similar to the value obtained previously in this lipid bilayer system [2–4].

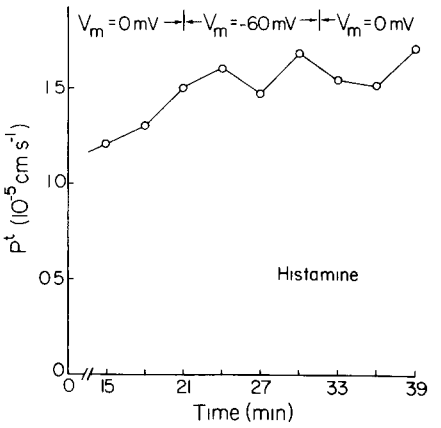

Fig. 1. Noneffect of membrane voltage (60 mV) on the one-way flux of histamine through a lipid bilayer membrane. The aqueous solutions were symmetrical and contained 80 mM NaCl, 20 mM Ches buffer (pH 9.8) and 10 μM histamine.

Fig. 3 shows the tryptamine permeability data, plotted according to Eqn. 2. Tryptamine fluxes were measured over a pH range of 7.5 to 10.0 at concentrations ranging from 5 to 10 μM. Linear regression analysis of the data gave a membrane permeability to nonionic tryptamine of $1.75 \cdot 10^{-1}$ cm $\cdot$ s^{-1} (Table I) and an unstirred layer permeability of $6.8 \cdot 10^{-4}$ cm $\cdot$ s^{-1}. The linearity of the relation between $1/P^t$ and $[B^t]/[B]$ indicates that only nonionic tryptamine is crossing the membrane as a significant rate. This was substantiated by the observation that a membrane voltage of 60 mV had no effect on the one-way flux of tryptamine at pH 7.5 (data not shown).

TABLE I

PERMEABILITY COEFFICIENTS OF HISTAMINE, THEOPHYLLINE AND TRYPTAMINE THROUGH LIPID BILAYER MEMBRANES

Solute	pK_a	P_B^m or P_{HA}^m [a] (cm $\cdot$ s^{-1})	P^t at pH 7.5 [b] (cm s^{-1})
Histamine	9.8	$(3.46 \pm 0.42) \cdot 10^{-5}$ (n = 4)	$1.73 \cdot 10^{-7}$
Theophylline	8.7	(2.92 ± 0.26) 10^{-4} (n = 7)	1.97 10^{-4}
Tryptamine	10.3	$(1.75 \pm 0.38) \cdot 10^{-1}$ (n = 7)	$1.70 \cdot 10^{-4}$

[a] P_B^m is the membrane permeability to nonionized base (histamine and tryptamine). P_{HA}^m is the membrane permeability to nonionized acid (theophylline) Values shown are the means ± S.D. (n = number of membranes), calculated by Eqn. 2.

[b] P^t is the total or 'effective' permeability, estimated for physiological conditions (pH 7.5) and taking into account the degree of ionization and the effects of chemical reactions in the unstirred layers. The values for theophylline and tryptamine are taken from the regression slopes in Figs 2 and 3.

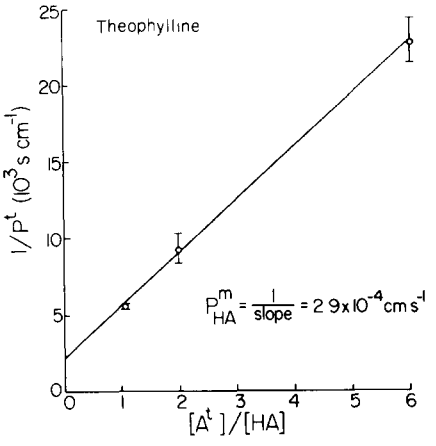

Fig. 2 Theophylline permeability as a function of pH (7.7 to 9.4), plotted according to Eqn. 2. The ratio $[A^t]/[HA]$ was calculated assuming a pK of 8.7 [9].

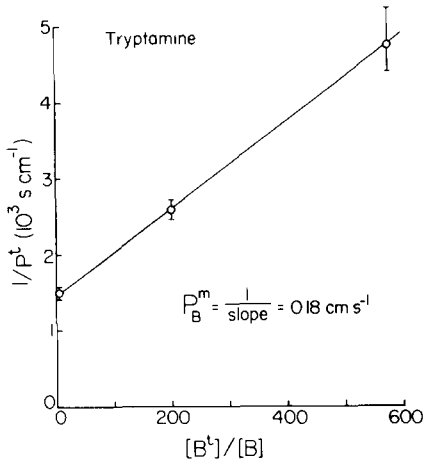

Fig. 3. Tryptamine permability as a function of pH (7.5 to 10.0), plotted according to Eqn. 2. The ratio $[B^t]/[B]$ was calculated assuming a pK of 10.3 [8].

Discussion

Table I summarizes the nonionic permeability coefficients for histamine, theophylline and tryptamine. Also shown are the total or 'effective' permeabilities under physiological conditions, i.e., pH 7.5. The values of P^t at pH 7.5 take into account the degree of ionization and the effects of chemical reactions in the unstirred layers as predicted by Eqns. 1 and 2. The unstirred layer reactions are especially important in tryptamine transport. For example, over the pH range of 7.5 to 10.0 the ratio $[B^t]/[B]$ changes about 300-fold but P^t changes less than 3-fold (Fig. 3). The reason for the relative insensitivity of P^t to $[B^t]/[B]$ is that HB^+ 'facilitates' tryptamine diffusion through the unstirred layers. Thus, P^t is much less sensitive to pH than would be predicted by the degree of ionization.

Fig. 4 shows schematic concentration profiles for nonionized and ionized tryptamine across the membrane and unstirred layers at pH 7.5. The gradients are calculated from the measured values of the net flux, P^m_B, P^{ul} and the assumption that pH is constant throughout the unstirred layer. At pH 7.5, more than 99% of the tryptamine crosses the unstirred layer as HB^+. As B diffuses through the membrane its concentration at the *cis* membrane surface is replenished by conversion of HB^+ to B. On the *trans* side of the membrane, more than 99% of B is converted to HB^+, which then diffuses into the bulk solution. The pH throughout the unstirred layer is maintained constant by the presence of impermeant buffer. At pH 7.5 about 70% of the concentration gradient occurs across the membrane and 30% across the unstirred layers. At higher pH the unstirred layer would become totally rate limiting. At lower pH the membrane would become totally rate limiting because HB^+ can diffuse through the unstirred layer much faster

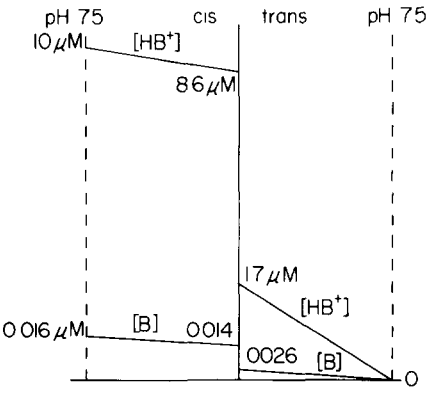

Fig. 4. Schematic concentration profiles for nonionic and ionic tryptamine diffusing across a lipid bilayer membrane and unstirred layers at pH 7.5. Aqueous solutions contained 90 mM NaCl, 10 mM Hepes, and 10 μM tryptamine (*cis* side only). Concentration gradients are not drawn exactly to scale.

than B can diffuse through the membrane, i.e., $P^{ul}[HB^+] >> P^m[B]$.

The permeability of lipid bilayer membranes to various weak acids and weak bases has been studied by Bean et al. [10] and Inui et al. [11]. Both groups observed that the total permeabilities (P^t) changed in the direction, but not the magnitude, predicted from the degree of ionization. This is shown graphically in Fig. 5, which shows that P^t for tryptamine is independent of pH at pH > 9 but proportional to pH when pH < 8. The change in slope at pH 8–9 is due to the fact that the unstirred layer is rate limiting at pH > 9, whereas the membrane is rate limiting at pH < 8. This nonlinear dependence of P^t on pH led Bean et al. and Inui et al. to the erroneous conclusion that both the ionic and nonionic forms of weak acids and bases are crossing the membrane.

Fig. 5 also shows the apparent nonionic tryptamine permeability (P_B^{app}) as calculated by the method of Bean et al., i.e., $P_B^{app} = P^t[B^t]/[B]$, where $[B^t] = [B] + [HB^+]$. At pH 6–7, P_B^{app} is similar to the true nonionic permeability (0.18 cm · s^{-1}), because the membrane is rate limiting. However, at pH > 8,

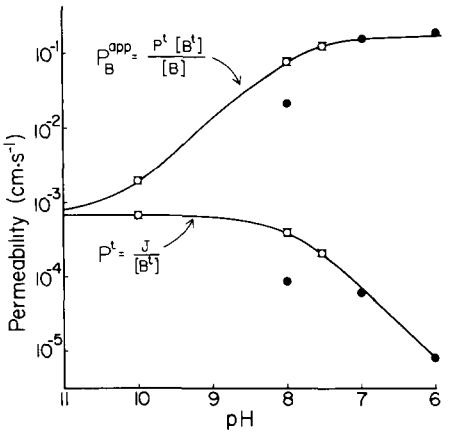

Fig. 5. Tryptamine permeability as a function of pH. P^t is the observed flux divided by the total tryptamine concentrations, i.e., $[B^t] = [B] + [HB^+]$. P_B^{app} is the apparent nonionic permeability calculated by the method of Bean et al. [10], i.e., P^t divided by the fraction of nonionized tryptamine. The open circles are our data, and the closed circles are the data of Bean et al., which we have recalculated assuming that pK = 10.3. Bean's data were obtained with sphingomyelin-tocopherol bilayers. Extrapolated values of P^t are calculated from Eqn. 2, assuming $P_B^m = 0.18$ cm · s^{-1} and $p^{ul} = 6.8 \cdot 10^{-4}$ cm · s^{-1}.

P_B^{app} is much less than the true nonionic permeability (P_B^m) because the unstirred layer is rate limiting. At pH 8 the permeability values of Bean et al. fall below the lines because they measured net tryptamine fluxes in solution buffered with 5 mM phosphate, which is not an effective buffer at pH 8 (pK = 6.8). Under these conditions, pH gradients develop in the unstirred layers and the net flux becomes partly limited by the rate at which H^+/OH^- can diffuse through the unstirred layers [2,3].

The membrane permeability to nonionic tryptamine (0.18 cm · s^{-1}) is one to two orders of magnitude higher than the permeability to auxin (indoleacetic acid) [4,10], which is structurally similar to tryptamine except that a carboxyl group replaces the $-CH_2NH_2$ in tryptamine. Serotonin (5-hydroxytryptamine) is structurally similar to tryptamine except for the addition of an -OH group. Thus, we would expect serotonin permeability to be at least two orders of magnitude lower than tryptamine permeability [12]. This expectation is supported by the data of Bean et al. [10], who observed a total serotonin permeability of $1.1 \cdot 10^{-6}$ cm · s^{-1} at pH 7.0. Assuming that their solutions were adequately buffered at pH 7 and that the serotonin permeability was rate limited by the membrane, we estimate the nonionic serotonin permeability of their lipid bilayer (brain phospholipid-tocopherol) membrane to be $7 \cdot 10^{-4}$ cm · s^{-1}. Schadt and Haeusler [13] reported that the serotonin permeability of a lipid bilayer (dioleoylphosphatidylcholine -decane) membrane was $1.4 \cdot 10^{-7}$ cm · s^{-1}. However, their solutions were unbuffered and pH was not reported. Thus, their data do not provide an estimate of the true serotonin permeability.

In isolated pancreatic islets both theophylline and caffeine equilibrate with a half-time of less than 1 min, and carrier mediated transport mechanisms have been postulated [14]. Although our results do not disprove this possibility, a carrier mediated transport seems unlikely because under physiological conditions the effective permeability is $>10^{-4}$ cm · s^{-1} (Table I), almost as high as the water permeability [15]. For example, if a hypothetical spherical cell had a diameter of 20 μm, the half-time for equilibration with external theophylline would be about 2 s. Thus, it seems unlikely, a priori, that interactions with a membrane 'carrier' would significantly

increase the rate of equilibration. However, chemical reactions in the unstirred layers can cause apparent saturation kinetics in weakly buffered solutions, because at high permeant concentrations the buffer capacity is exceeeded and the net flux becomes limited by the rate at which H^+/OH^- can diffuse through the unstirred layer [2,3]. A knowledge of such unstirred layer effects is important in designing experiments to test for saturable transport mechanisms. Furthermore, high (millimolar) concentrations of pharamacological agents which rapidly cross cell membranes, e.g., tryptamine, may alter intracellular pH and thus cause secondary (indirect) effects on membrane permeabilities to other solutes, e.g., Na^+ and Cl^- [16].

Recently Ersinkyan et al. [17] reported that histamine diffuses through lipid bilayer (phosphatidylcholine-cholesterol) membranes as a singly charged cation at pH 7.3. However, their electrical conductance measurements did not allow an estimation of the permeability to nonionic histamine. At 1 mM histamine they observed a total membrane conductance (G_m) of about 10^{-9} S · cm^{-2}. Thus, we can estimate the maximum permeability to ionic histamine (P_{HB^+}) from the equation, $P_{HB^+} \leqslant RTG_m/z^2F^2$ [HB^+], where R is the gas constant, T is the absolute temperature, z is the ionic valence, and F is the Faraday constant [18]. This calculation yields a value $P_{HB^+} \leqslant 2.7 \cdot 10^{-10}$ cm · s^{-1}, about three orders of magnitude lower than our value of P^t at pH 7.5 (Table I). Thus, we believe that the net histamine flux they observed at pH 7.3 was actually due to nonionic diffusion of histamine.

In human erythrocytes, histamine uptake at pH 7.5 appears to occur by simple diffusion [19]. From the half-time for equilibration (approx. 10 min) we estimate the rate constant (k) to be $1.2 \cdot 10^{-3}$ s^{-1}, assuming that histamine uptake follows first-order kinetics. The permeability coefficient (P^t) is equal to KV/A, where V/A is the volume/surface ratio of the cell, i.e., $6.1 \cdot 10^{-5}$ cm [20]. Thus, P^t is about $7.1 \cdot 10^{-8}$ cm · s^{-1}, roughly similar to our value of P^t at pH 7.5 (Table I). This agreement is not surprising because the nonelectrolyte permeabilities of phosphatidylcholine-cholesterol bilayers and mammalian erythrocytes are generally similar within a factor of five [21–23].

Acknowledgements

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