Uptake of Imipramine in Rat Liver Lysosomes In Vitro and Its Inhibition by Basic Drugs

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ABSTRACT

We investigated the uptake of imipramine (IMP) in highly purified lysosomes from rat liver and its inhibition by a variety of basic drugs in vitro. The uptake of [3H]IMP into lysosomes peaked in less than 20 s, showing little temperature dependency or countertransport phenomena. It was accelerated by increase of extralysosomal pH, stimulated by Mg2+-ATPase, and suppressed by acidic ionophores. However, the uptake of [3H]IMP into lysosomes was approximately 140-fold higher than the value expected from the pH-partition theory. IMP and other weak lipophilic bases like chlorpromazine and propranolol raised the intralysosomal pH, and their potency was stronger than that of NH4Cl, a typical pH-perturbing weak base. A variety of basic drugs inhibited the uptakes of [3H]IMP and [14C]methylamine into lysosomes, their 50% inhibitory concentrations (IC50) being almost the same for [3H]IMP and [14C]methylamine uptake (r = 0.842). A high correlation (r = 0.946) was observed between the IC50 values (for the inhibition of [3H]IMP uptake) and the lipophilicity (Poct values). These results suggest that the accumulation of lipophilic basic drugs is driven primarily by the transmembrane pH difference (pH-partition theory) but with the involvement of some additional mechanism(s) related to drug lipophilicity, possibly binding (partition or adsorption) to lipophilic substance(s) and/or aggregation within lysosomes. Based on this idea, we have established a model that described and successfully simulated the weak base-induced pH increase, the accumulation of a lipophilic weak base (IMP), and the inhibition of accumulation of IMP by lipophilic basic drugs.

Lipophilicity of basic drugs has been shown to be the primary determinant of their tissue distribution, and their hepatic accumulation increases with increasing lipophilicity, being especially pronounced in mitochondria (Proost et al., 1997). However, we have shown that 1) lipophilic basic drugs [imipramine (IMP), biperiden, and chlorpromazine (CPZ)] have large tissue distributions (Yokogawa et al., 1990a,b, 1992), with lysosomes accounting for approximately 10% of the total distribution in rat liver, and 2) the lysosomal contribution increases as the plasma drug concentration decreases (Ishizaki et al., 1996). Furthermore, we have shown that the affinity of several lipophilic basic drugs for tissue is decreased to 20 to 80% by NH4Cl treatment, suggesting a significant contribution of lysosomes to the distribution of basic drugs (Ishizaki et al., 1998).

Although the potential role of lysosomes in tissue distribution of basic drugs has been well documented (de Duve et al., 1974; Ohkuma and Poole, 1981; MacIntyre and Cutler, 1988), the uptake mechanism of these drugs into lysosomes remains to be established in detail. Basic drugs are generally thought to enter cells by diffusion and to accumulate as cations (protonated bases) inside acidic vacuolar compartments (de Duve et al., 1974; Reijngoud and Tager, 1976). Poole and Ohkuma (1981) found that weakly basic substances cause a concentration-dependent increase in the intralysosomal pH as well as cellular vacuolation, both of which are probably associated with the accumulation of the drugs. Furthermore, Ohkuma and Takano (1997) established an in vitro cell-free system for the assessment of the effect of basic drugs on the intralysosomal pH, as well as vacuolation. However, more precise studies are required to clarify the mechanism of uptake of certain (lipophilic) basic drugs in lysosomes.

The mechanism of distribution of basic drugs is also important from the point of view of combination therapy with basic drugs, where changes of pharmacokinetic disposition are expected to occur as a result of competition for uptake

ABBREVIATIONS: IMP, imipramine; AMA, amantadine; ATR, atropine; BAF, bafilomycin A1; CPZ, chlorpromazine; CQ, chloroquine; ΔpH, transmembrane pH gradient; DTZ, diltiazem; FO, fluorescein isothiocyanate-dextran; MeNH2, methylamine; NiG, nigericin; Poc, octanol-water partition coefficient of the nondenized drug; QN, quinine; PPR, propranolol; TFP, trifluoperazine; TMAH, tetramethylammonium hydroxide; V-ATPase, vacuolar-type H+-ATPase; VP, verapamil.
into lysosomes, as we reported previously (Ishizaki et al., 1996). In clinical treatment, adverse effects have been reported in some cases of combination treatment with chloroquine (CQ) (antimalarial lysosomal inhibitor) and IMP (or desipramine) (basic tricyclic antidepressants) (Bitonti et al., 1988; Onyeji et al., 1993): Onyeji et al. (1993) reported no apparent pharmacokinetic interaction between CQ and IMP, whereas Bitonti et al. (1988) reported that several basic antidepressants reverse CQ resistance and that one of the mechanisms of the interaction might involve lysosomotropic effects. Several other authors have reported that the accumulation of basic drugs in lysosomes should not be clinically ignored because it induces side effects by impairing phospholipid metabolism (Honegger et al., 1993), induces pharmacokinetic interactions (Daniel and Wójcikowski, 1997), and is sometimes associated with the appearance and duration of some pharmacological actions (Antone et al., 1995).

In this article, we deal with the mechanism of uptake of a lipophilic base (IMP) into lysosomes and its inhibition by various basic drugs by using highly purified lysosomes isolated from rat liver. We also present a mathematical model that successfully describes these phenomena.

**Experimental Procedures**

**Materials.** Fluorescein isothiocyanate-dextran (FD; average molecular weight 70,000), anti-fluorescein rabbit IgG (heavy plus light) fraction, and Percoll were purchased from Sigma (St. Louis, MO), Molecular Probes Inc. (Eugene, OR), and Pharmacia (Uppsala, Sweden), respectively. CPZ, trifluoperazine (TFP), IMP, quinine (QN), verapamil (VP), diltiazem (DTZ), propranolol (PPr), CQ, amantadine (AMA), and atropine (ATP) were obtained from Sigma. Tributylamine was obtained from Merck (Darmstadt, Germany). [3H]IMP (24 Ci/mmol), [3H]Hinulin (1.65 Ci/mmol), and [14C]sucrose (580 mCi/mmol) were obtained from Amersham International Ltd. (Bucks, UK), and [14C]methylamine ([14C]MeNH2; 51.8 mCi/mmol) hydrochloride was obtained from DuPont NEN (Boston, MA). All other chemicals were of reagent grade and were used without further purification.

**Animals.** Male Wistar rats (260 ± 25 g; mean ± S.D.) were obtained from Sankyo Labo Service (Sankyo Laboratory Animal) Co. (Toyama, Japan).

**Preparation of Rat Liver Lysosomes.** The rat liver lysosomes were isolated essentially according to Arai et al. (1991). Briefly, the rats were first injected i.p. with FD at a dose of 100 mg/100 g of body weight, and starved overnight. The excised livers were perfused with ice-cold 0.25 M sucrose and homogenized with 4 volumes of ice-cold 0.25 M sucrose. All subsequent steps were performed at 4°C. The homogenate was centrifuged twice at 340 g for 5 min. The resulting postnuclear supernatant was then incubated at 37°C for 5 min in the presence of 1 mM CaCl2 to swell mitochondria and centrifuged at 10,000 g for 30 min. The resulting pellet was resuspended in isoosmotic Percoll (0.25 M sucrose) at a density of 1.075 g/ml (pH 7.4) and centrifuged at 60,000 g for 15 min. The lysosomal fractions were pooled and centrifuged at 100,000 g for 1 h. The broad turbid layer was collected, diluted with 10 volumes of 0.25 M sucrose, and centrifuged at 10,000 g for 30 min. The pellets were washed under the same conditions to remove the Percoll and resuspended in chilled buffer.

**Determination of Intralysosomal pH.** The intralysosomal pH was determined fluorometrically based on the pH sensitivity of the fluorescence spectrum (and intensity) of FD accumulated within lysosomes according to the method of Ohkuma and Poole (Ohkuma and Poole, 1978; Ohkuma, 1989). Briefly, the lysosomal fraction (100 μg of protein) was incubated in 20 mM HEPES-tetramethylammonium hydroxide (TMAH) (pH 7.4) containing 0.2 M sucrose and 2 mg/ml BSA, and fluorescence was determined in a spectrophotometer (Hitachi 650-40K; Hitachi, Tokyo, Japan) at 25°C with excitation and emission wavelengths of 495 and 550 nm, respectively. The intralysosomal pH was estimated from the ratio of fluorescence produced by excitation at 495 nm to that produced by excitation at 450 nm, at an emission wavelength of 520 nm, after subtraction of the fluorescence of extralysosomal FD.

**Uptake of [3H]IMP and [14C]MeNH2.** Uptake of [3H]IMP (1 μM, 0.025 μCi) into lysosomes (65–80 μg of protein) was determined at 4, 25, or 37°C, either by a centrifugation method or a rapid filtration method. In the centrifugation method, the samples (1 ml) were centrifuged at 12,300 g for 2 min (4°C), and in the rapid filtration method, the samples were filtered through Whatman GF/B glass filters (Whatman Inc., Clifton, NJ). The radioactivity of the supernatant (centrifugation method) or the filter paper (rapid filtration method) was determined in scintillation cocktail (ACS-II; Amersham Corp., Arlington Heights, IL) by using a liquid scintillation counter (Aloka LSC-3600; Aloka, Tokyo, Japan). Correction for [3H]IMP in the extralysosomal space on the glass filter was done by the use of [14C]sucrose added to the buffer. In some experiments, lysosomes were preincubated with nigericin (NIG; 2.5 μM), ATP (1 mM), and/or bafilomycin A1 (BAF; 10 nM) for 3 min at 4 or 37°C before addition of [3H]IMP.

The uptake of [14C]MeNH2 (1 μM) was determined by the centrifugation method of Reijngoud and Tager (Reijngoud and Tager, 1973, 1976; Reijngoud, 1978) after a 1-h incubation at 4°C, and correction for extralysosomal [14C]MeNH2 was done by the use of [3H]hinulin added simultaneously to the assay buffer. Briefly, lysosomes (200 μl) were transferred to another tube (Bio-BIK, 0.4 ml; INA-OPTIKA Co., Osaka, Japan) containing silicon oil (d = 1.024, 50 μl), covered with 1% SDS containing 50% glycerol (50 μl), and centrifuged at 12,300 g for 5 min at 4°C. The tubes were frozen in liquid nitrogen. The part of the frozen tube containing the sample was cut out, placed in a vial containing scintillation cocktail, and kept at room temperature for 12 h; then the radioactivity was determined in a liquid scintillation counter.

**[3H]IMP Countertransport.** In uptake experiments, 800-μl aliquots of lysosomal fraction (100 to 130 μg of protein) were preincubated at 37°C with unlabeled IMP (100 μM) for 10 min, and then [3H]IMP was added (final 1 nM in 1 ml). In efflux experiments, each lysosomal fraction was preincubated at 37°C with [3H]IMP (1 nM) for 10 min and then diluted to 1 ml with buffer containing unlabeled IMP (100 μM). Samples were collected 2.5, 5, 10, 15, 20, and 30 s after dilution, and the radioactivity in lysosomes was assessed as described above.

**Inhibition of Uptake of [3H]IMP and [14C]MeNH2.** To examine the effect of basic drugs and NH4Cl on the uptake of [3H]IMP and [14C]MeNH2, the lysosomes were preincubated in the presence or absence of the drugs at 4°C for 10 or 60 min before the addition of [3H]IMP (1 nM) or [14C]MeNH2 (1 μM), respectively. The uptake of [3H]IMP and [14C]MeNH2 was determined as described above after 10 and 60 min, respectively.

**Determination of Lysosomal Volume.** The lysosomal volume was determined from the volume of the lysosomal pellet (as detected by using [3H]H2O) by subtraction of extralysosomal volume (as detected by using membrane-impermeable [14C]sucrose) according to Reijngoud and Tager (Reijngoud and Tager, 1973, 1976; Reijngoud, 1978). Briefly, lysosomes were incubated with [3H]H2O and [14C]sucrose at 4°C for 10 or 60 min after the addition of [3H]IMP (1 nM) or [14C]MeNH2 (1 μM), respectively. The uptake of [3H]IMP and [14C]MeNH2 was determined as described above after 10 and 60 min, respectively.

**Lipophilicity of Drugs.** Drug lipophilicity was determined according to Yokogawa et al. (1990b). Briefly, octanol was used as an
organic solvent, and isotonic phosphate buffer (pH 7.4) was used as an aqueous solution. An exact amount (3–100 ml) of each solution was transferred to a siliconized glass-stoppered flask and shaken for 16 h at 37°C to achieve complete equilibrium. After centrifugation at 3000 rpm for 10 min, the amount of base in the aqueous phase was determined by gas chromatography. The apparent partition coefficients were obtained by dividing the concentration of the drug in the organic phase by that in the aqueous phase, and the (true) octanol-water partition coefficients of the nonionized form of the basic drugs (P0/w) were calculated using the pKaw values (shown in Table 1) and the Henderson-Hasselbalch equation.

**Determination of Drugs.** CPZ, IMP, VP, TFP, and CQ were determined according to Yokogawa et al. (1990b), and ATR and AMA were determined according to Briggs and Simons (1983) and Siouffi and Pommier (1980), respectively, all by gas chromatography. QN, DTZ, and PPR were determined by measuring their UV absorbance.

**Determination of Protein.** Proteins were determined by a Coomassie Brilliant Blue/liquid phase method using a commercial protein assay kit (Bio-Rad Laboratories Ltd., Osaka, Japan).

**Data Analysis.** The 50% inhibitory concentrations (IC50) of basic drugs were determined from the best-fit curves using logit-log regression (Rodbard, 1974). The parameters were estimated by the least-squares method using the MULTI program (Yamaoka et al., 1981).

**Results**

**Uptake and Efflux of [3H]IMP by Lysosomes.** Figure 1A shows the time courses of initial uptake of [3H]IMP (1 μM) in lysosomes in salt-free buffer (pH 7.4) at 4, 25, and 37°C. The initial uptake rate showed little temperature dependence, although the plateau levels of uptake (reached within 20 s) were slightly higher at higher temperatures. However, the [3H]IMP accumulated within lysosomes subsequently decreased to approximately 95, 60, and 35% of the plateau levels at 4, 25, and 37°C, respectively, at 60 min (Fig. 1B). These decreases in the amount of [3H]IMP accumulated within lysosomes were associated with increases in intralysosomal pH to 5.5, 5.9, and 6.5 (at 60 min) at 4, 25, and 37°C, respectively.

Figure 2 shows the time courses of the uptake of [3H]IMP (1 μM) in KCl (0.1 M) buffer (pH 7.4) and the effects of ATP (1 mM), BAF (10 nM) and NIG (2.5 μM). At 37°C (Figure 2A), [3H]IMP accumulated within lysosomes decreased more rapidly than in KCl-free buffer, again in association with an increase in pH (data not shown). In the presence of ATP, the uptake was clearly higher than that of the control (–ATP); it showed little decrease over 20 min and was not associated with an increase in pH (data not shown). Addition of BAF [a specific vacuolar-type H+-ATPase (V-ATPase) inhibitor that abolishes active proton transport] decreased the uptake of [3H]IMP almost to the control level, and NIG [a H+/K+-exchanging ionophore that abolishes transmembrane pH gradient (ΔpH)] completely abolished the uptake. At 4°C (Fig. 2B), the uptake level of [3H]IMP hardly changed in 20 min even in the absence of ATP but was decreased to 30% of the control by NIG. In the following studies, most experiments were performed in energy-free buffer to avoid possible secondary effects of inhibition of V-ATPase.

The countertransport effects on IMP were also examined at pH 7.4 at 37°C to elucidate the mechanism of transport of IMP through the lysosomal membrane. As shown in Fig. 3, a significant difference was hardly observed in the uptake (A) or the efflux (B) of [3H]IMP (1 nM) in the presence and absence of IMP (100 μM) in the trans-side of lysosomal membranes.

**Effect of Extralysosomal pH on the Uptake of [3H]IMP into Lysosomes.** Figure 4 shows the effect of extralysosomal pH on the lysosomal uptake of [3H]IMP (1 μM) at 4°C in 10 min. The uptake of [3H]IMP increased gradually with increasing alkalization of the extralysosomal environment. The uptake at pH 8.5 was almost 11 times higher than that at pH 5.0.

**Effect of Lipophilic Weak Bases on the Intralysosomal pH.** Figure 5 shows the effects of lipophilic weak bases (CPZ, IMP, and PPR) on the internal pH of lysosomes. All the bases raised the intralysosomal pH dose dependently, and their effective concentrations were in the order of CPZ < IMP < PPR < NH4Cl.

**Inhibition of [14C]MeNH2 or [3H]IMP Uptake by Basic Drugs.** Figure 6 shows the inhibition of the uptake of [3H]IMP and [14C]MeNH2 into lysosomes by CPZ and PPR compared with that by NH4Cl. Uptake of [3H]IMP was inhibited by these bases in parallel with the uptake of [14C]MeNH2, showing similar dose-response relationships. However, the 50% inhibitory concentrations (IC50) values of CPZ and PPR for the uptake of [3H]IMP and [14C]MeNH2 were lower (approximately 1/107 and 1/13, respectively) than those of NH4Cl.

Table 1 summarizes the pKaw values, the log Poct, and the IC50 values (for the inhibition of uptake of [3H]IMP and [14C]MeNH2) of the weak basic drugs used in this study. The IC50 values range from 8.47 μM to 3.35 mM, but they are...
similar for the uptakes of \( [3\text{H}]\text{IMP} \) and \( [14\text{C}]\text{MeNH}_2 \) (correlation coefficient, \( r = 0.842 \)), as shown in Fig. 7. There was also a good inverse correlation (correlation coefficient, \( r = 0.946 \)) between the IC \(_{50}\) and the \( P_{\text{oct}} \) values of the basic drugs, except for dibasic CQ, which mostly takes a diprotinated form in the physiological pH range (5–7) (Fig. 8).

Possible Mechanisms of Massive Uptake of Lipophilic Basic Drugs in Lysosomes. Because the lipophilic basic drugs (CPZ, IMP, and PPR) increased the intralysosomal pH at lower concentrations than did \( \text{NH}_4\text{Cl} \) and their concentration ratios between lysosomes and extralysosomal buffer were 1 or 2 orders of magnitude higher than that of \( \text{MeNH}_2 \) or \( \text{NH}_4\text{Cl} \) (Fig. 5), it was assumed that additional mechanisms, such as binding to lipidic constituents (such as membrane) and/or aggregation within lysosomes, contributed to the uptake of these drugs. To explore these possibilities, the effects of NIG, ATP, and BAF on \( [3\text{H}]\text{IMP} \) uptake into lysosomes at 37°C (A) and 4°C (B) in KCl medium were examined (Fig. 2). Lysosomal fractions were preincubated at 37°C (A) or 4°C (B) for 3 min with NIG (2.5 \( \mu \text{M} \)), ATP (1 \( \text{mM} \)), and/or BAF (10 \( \text{nM} \)) before addition of \( [3\text{H}]\text{IMP} \) (1 \( \mu \text{M} \)). The values presented are the means ± S.D. of the three experiments. ○, control; ●, +NIG (2.5 \( \mu \text{M} \)); ▲, +ATP (1 \( \text{mM} \)); ■, +ATP (1 \( \text{mM} \)) + BAF (10 \( \text{nM} \)). Buffer: 0.1 M KCl, 0.2 M sucrose, 10 mM MgCl\(_2\), HEPES-TMAH (pH 7.4).

Fig. 2. Effects of NIG, ATP, and BAF on \( [3\text{H}]\text{IMP} \) uptake into lysosomes at 37°C (A) and 4°C (B) in KCl medium. Lysosomal fractions were preincubated at 37°C (A) or 4°C (B) for 3 min with NIG (2.5 \( \mu \text{M} \)), ATP (1 \( \text{mM} \)), and/or BAF (10 \( \text{nM} \)) before addition of \( [3\text{H}]\text{IMP} \) (1 \( \mu \text{M} \)). The values presented are the means ± S.D. of the three experiments. ○, control; ●, +NIG (2.5 \( \mu \text{M} \)); ▲, +ATP (1 \( \text{mM} \)); ■, +ATP (1 \( \text{mM} \)) + BAF (10 \( \text{nM} \)). Buffer: 0.1 M KCl, 0.2 M sucrose, 10 mM MgCl\(_2\), HEPES-TMAH (pH 7.4).

Fig. 3. Countertransport effect on IMP uptake (A) and efflux (B) in lysosomes at 37°C. A, lysosomal fractions were preincubated with unlabeled IMP (100 \( \mu \text{M} \)) for 10 min before addition of \( [3\text{H}]\text{IMP} \) (1 \( \text{nM} \)). The values presented are percentages relative to the equilibrium values. B, lysosomal fractions were preincubated with \( [3\text{H}]\text{IMP} \) (1 \( \text{nM} \)) for 10 min before addition of unlabeled IMP (100 \( \mu \text{M} \)). The values presented are percentages relative to the zero-time value, expressed as the means of three experiments. ○, control; ▲, +IMP (100 \( \mu \text{M} \)). Buffer: 0.1 M KCl, 0.2 M sucrose, 10 mM MgCl\(_2\), HEPES-TMAH (pH 7.4).

Fig. 4. Effect of extralysosomal pH on \( [3\text{H}]\text{IMP} \) uptake into lysosomes. The uptake of \( [3\text{H}]\text{IMP} \) (1 \( \mu \text{M} \)) into lysosomes was determined at various values of extralysosomal pH (at 4°C in 10 min). The bars present the means ± S.D. of three experiments. Buffer: 0.3 M sucrose, 2 mg/ml BSA.

Fig. 5. Effect of the weakly basic drugs on the intralysosomal pH. ▲, CPZ; △, IMP; ○, PPR; ●, \( \text{NH}_4\text{Cl} \). Buffer: 0.3 M sucrose, 2 mg/ml BSA, 20 mM HEPES-TMAH (pH 7.4) at 25°C. The dotted line (— — —), the broken lines (– – –), and the solid lines show simulation curves for \( \text{NH}_4\text{Cl} \) using eq. 1; for CPZ, IMP, and PPR using eq. 2 for binding; and for CPZ, IMP, and PPR using eq. 3 for aggregation, all obtained by means of the MULTI program.

Fig. 6. Drug concentration (M) in medium

Fig. 4. Effect of extralysosomal pH on \( [3\text{H}]\text{IMP} \) uptake into lysosomes. The uptake of \( [3\text{H}]\text{IMP} \) (1 \( \mu \text{M} \)) into lysosomes was determined at various values of extralysosomal pH (at 4°C in 10 min). The bars present the means ± S.D. of three experiments. Buffer: 0.3 M sucrose, 2 mg/ml BSA.
somites, must be at work, as shown in Fig. 9. This would shift the equilibrium so that larger amounts of lipophilic bases accumulate and increase the pH within lysosomes, eventually inhibiting the uptake of [3H]IMP and [14C]MeNH₂ at relatively low concentrations compared with NH₄Cl. NH₄Cl and MeNH₂ do not show such phenomena (Reijngoud and Tager, 1973, 1976; Poole and Ohkuma, 1981).

On the basis of these considerations, we tried to simulate the increase of the intralysosomal pH, the accumulation of basic drugs, and the competition for accumulation by the other lipophilic basic drugs. First, the buffering capacity (β) of lysosomes was estimated from the relationship between the concentration of NH₄Cl (x) and the lysosomal pH (y) at medium pH 7.4 (Fig. 5), by a nonlinear least-squares method using the MULTI program, based on the following equation (eq. 1, see Appendix):

\[ x = \beta \cdot (y - N) \cdot \frac{10^{-7.4}}{10^{-y}} \]

where \( N \) represents the original lysosomal pH (~5.5) in the absence of drug. The calculated value of \( N \) was 46 ± 3 (mM/\( \text{pH} \), mean ± S.D.). The fitting curve produced by using the \( \beta \)-value is also shown in Fig. 5 and fits well with the observed values. For the other lipophilic bases (CPZ and PPR), either eq. 2 (for binding, \( L \) = a proportional constant; see Appendix) or eq. 3 (for aggregation; see Appendix) was applied to the relation between drug concentration (x) and lysosomal pH (y) at medium pH 7.4, and the \( K_x \) (for binding) or \( K_z \) (for aggregation) value was obtained by the MULTI program:

\[ x = \frac{\beta \cdot (y - N)}{1 + K_1 \cdot L} \cdot \frac{10^{-7.4}}{10^{-y}} \]  

(2)

\[ x = \sqrt{1 + 4 \cdot K_z \cdot \beta \cdot (y - N) - 1} \cdot \frac{10^{-7.4}}{2 \cdot K_z} \]  

(3)

The \( K_x \) and \( K_z \) values for CPZ, IMP, and PPR, as well as the \( \beta \)-value, are shown in Table 2, and the fitting curves based on these values are shown in Fig. 5. Table 2 also shows the Akaike's information criterion values for the three drugs; the values are slightly smaller for the “binding” than for the “aggregation” curve. Figure 6 shows the fitting curves, produced based on these values, to the inhibitory effects of NH₄

### Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>pK_{a}^a</th>
<th>Log ( P_{oct}^c )</th>
<th>( \text{IC}_{50}^a ) [3H]IMP</th>
<th>( \text{IC}_{50}^a ) [14C]MeNH₂</th>
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<tr>
<td>CPZ</td>
<td>9.3</td>
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<td>4.77</td>
<td>2.17 × 10⁻⁴</td>
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<td>2.28</td>
<td>4.79 × 10⁻⁴</td>
<td>1.18 × 10⁻⁴</td>
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<tr>
<td>VP</td>
<td>7.7</td>
<td>3.13</td>
<td>5.47 × 10⁻⁴</td>
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</tr>
<tr>
<td>DTZ</td>
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<td>3.1</td>
<td>6.36 × 10⁻⁵</td>
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</tr>
<tr>
<td>PPR</td>
<td>8.4, 10.8</td>
<td>5.82</td>
<td>9.43 × 10⁻⁵</td>
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<td>2.30 × 10⁻⁴</td>
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<td>1.53</td>
<td>1.48 × 10⁻⁴</td>
<td>6.24 × 10⁻⁴</td>
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</table>

TBA, tributylamine.

a The values of 50% inhibitory concentration (IC₅₀) calculated using the best-fit curve by logit-log regression (Rodbard, 1974). The IC₅₀ values were determined under the conditions given in the legend to Fig. 6.

b Values were obtained from Reynolds (1989).

c Logarithm of the octanol-water partition coefficient of the nonionized form of the drug (see Experimental Procedures).

d Values were obtained from Hasegawa et al. (1984).
Cl (applying eq. 4; see Appendix), CPZ, and PPR [both applying either eq. 5 (for binding) or eq. 6 (for aggregation); see Appendix] on the uptake of $^{14}$CMeNH$_2$ and $^3$HIMP:

\[
x = -\frac{\beta}{10^{\gamma_4-N_1}} \frac{\log Z}{Z}
\]

(4)

\[
x = -\frac{\beta}{(1 + K_1 \cdot L) \cdot 10^{\gamma_4-N_1}} \frac{\log Z}{Z}
\]

(5)

\[
x = \frac{\sqrt{[1 + 4 \cdot K_2 \cdot \beta \cdot (-\log Z)] - 1}}{2 \cdot K_2 \cdot 10^{\gamma_4-N_1} \cdot Z}
\]

(6)

Table 3 summarizes the correlation coefficients between the observed and the calculated values for the inhibitory effects of the three basic drugs on the uptake of $^{14}$CMeNH$_2$ and $^3$HIMP. High correlation coefficients were obtained for NH$_4$Cl, as well as for CPZ and PPR.

**Fig. 7.** The relationship between the IC$_{50}$ values of various drugs for inhibition of the uptakes of MeNH$_2$ (1 $\mu$M) and IMP (1 $\mu$M).

**Fig. 8.** The relationship between the log $P_{oct}$ values of basic drugs and the IC$_{50}$ values for inhibition of the uptake of $^3$HIMP. The continuous line is the regression line for IC$_{50}$ and log $P_{oct}$. CQ has been excluded from the correlation because of its diprotonable nature with low $K_a$ values ($10^{-8}$ and $10^{-10}$).

**Fig. 9.** Diagrammatic representation of lysosomal accumulation of basic drugs through protonation, aggregation, and binding to membranes. B and BH$^+$ denote neutral and protonated species of a basic drug, respectively, and D$_{2+}$ denotes dimer of BH$^+$.

**Fig. 10.** Concentration dependency of accumulation of Basic Drug into Lysosomes. Figure 10 shows the concentration dependency of the accumulation of IMP within lysosomes. The concentration ratio (lysosomes/buffer, $F$) decreased with increasing concentration of IMP in the buffer. Figure 10 also shows the simulation curves calculated from eq. 7 (for binding; see Appendix) and eq. 8 (for aggregation; see Appendix):

\[
F = (1 + K_1 \cdot L) \cdot \frac{[H^+]_{in}}{[H^+]_{out}}
\]

(7)

\[
F = \left(1 + K_2 \cdot C_{out}\right) \frac{[H^+]_{in}}{[H^+]_{out}} \left(\frac{[H^+]_{in}}{[H^+]_{out}}\right)
\]

(8)

The curve based on the binding hypothesis showed a fairly good fit, whereas the one based on the aggregation hypothesis departed markedly from the observed values at lower concentrations ($<10 \mu$M) and showed a maximum at approximately 20 $\mu$M. These results suggest that the intralysosomal accumulation of lipophilic weakly basic drugs and the elevation of pH are influenced strongly by hydrophobic binding to lipidic constituents within lysosomes rather than aggregation of the drug molecules, at least at lower external concentrations.

**Discussion**

In this study, we examined in detail the IMP uptake into lysosomes and the competitive effects of various lipophilic, weakly basic drugs. Even in the absence of an energy source at 4°C, the uptake of $^3$HIMP into lysosomes reached a maximum very rapidly, and we observed little temperature dependency of initial uptake (Fig. 1A) or countertransport phenomena (Fig. 3), although the uptake was dependent on external pH (Fig. 4). Possible participation of $\Delta$H-independent simple adsorption of $^3$HIMP on external membranes of lysosomes can be ruled out because the uptake of $^3$HIMP...
was almost totally suppressed by NIG. Nevertheless, the \[^{3}H\]IMP accumulation within lysosomes decreased with time from the maximum values in a temperature-dependent manner (Fig. 1B). This was not due to lysosomal damage, which was minimal even at 37°C, judging from the release of lysosomal N-acetyl-D-glucosaminidase activity (data not shown), but was associated with an increase of the intr lysosomal pH. Based on these findings, we suggest that the temperature-dependent efflux reflects the increase of intr lysosomal pH (decreased \(\Delta pH\)) after a permeability increase of lysosomes to ions (e.g., tetramethylammonium and/or proton) at high temperatures. In KCl (a more physiological condition), this tendency was accelerated due to additional exchange of external K\(^+\) with internal H\(^+\). In KCl, however, ATP produced not only acceleration, but also prolongation, of the uptake of \[^{3}H\]IMP (little decrease of the accumulated \[^{3}H\]IMP was observed in 20 min at 37°C) because the intralysosomal pH was kept low due to continued supply of H\(^+\) by the V-ATPase on lysosomal membranes (Okamura and Takano, 1997). V-ATPase-driven lysosomal uptake has been reported for a variety of chemicals, including cations [e.g., tetraethylammonium; H\(^+\)-coupled antiporter-mediated (Moseley and van Dyke, 1995)] and weakly basic drugs such as tacrine (a drug used in the therapy of Alzheimer’s disease) (Antone et al., 1995) and daunomycin (an antineoplastic anthracycline antibiotic, carrier-nondependent) (Moriyama et al., 1994). We suggest that \[^{3}H\]IMP accumulates within lysosomes by a process of simple diffusion coupled with protonation within lysosomes due to lysosomal acidity maintained either by a Donnan-type equilibrium (Reijngoud and Tager, 1973; Reijngoud, 1978) or by an ATP-dependent proton pump (Okuma et al., 1982), although a possible role of some specific carrier(s) is not totally excluded.

de Duve et al. (1974) reported that the lysosomal uptake of basic drugs depends on the intralysosomal pH, and the concentration ratio (intralysosomal/extralysosomal) of the basic drug should be almost equal to the ratio of H\(^+\) ion concentration within lysosomes and extralysosomal space. In fact, the uptake of MeNH\(_2\) (or dibasic CQ) is generally accepted to depend only on the pH gradient and is used as an indicator of intravesicular pH (Reijngoud and Tager, 1973, 1976). The uptake of \[^{3}H\]IMP into lysosomes also depended on the pH gradient (Fig. 4). However, the concentration ratio of weak bases at medium pH 7.4 should theoretically be approximately 80 if we assume a value of 5.5 for the intralysosomal pH. Actually, the concentration ratios of \[^{3}H\]IMP and \[^{14}C\]MeNH\(_2\) at pH 7.4 differed by 2 orders of magnitude.

### TABLE 2
**Parameter values for the relationship between lysosomal pH and drug concentration**

<table>
<thead>
<tr>
<th>Drug</th>
<th>(K_a)</th>
<th>(AIC^b)</th>
<th>(K_a)</th>
<th>(AIC^b)</th>
<th>(K_a)</th>
<th>(AIC^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPZ</td>
<td>2.25 ± 0.16</td>
<td>−32.4</td>
<td>0.932 ± 0.067</td>
<td>−32.5</td>
<td>0.208 ± 0.022</td>
<td>−22.1</td>
</tr>
<tr>
<td>IMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH(_4)Cl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45.9 ± 2.5</td>
</tr>
</tbody>
</table>

*The partition (affinity) coefficient of the drug for lipidic substances of lysosomes (\(\mu g\) of protein \(^{-1}\); see eq. 14 in the text).*

*Akaike’s information criterion.*

*The dimerization constant of the drug (\(mM^{-1}\); see eq. 22 in the text).*

*The buffering capacity of lysosomes (\(mM/pH\); see eq. 11 in the text).*

### TABLE 3
**Measures of fit between the observed and model-predicted inhibitory effects of NH\(_4\)Cl, CPZ, and PPR on the uptake of \[^{3}H\]IMP and \[^{14}C\]MeNH\(_2\).**
The correlation coefficients (\(r\)) between the observed and calculated values for the inhibitory effects of the three basic drugs on the uptake of \[^{14}C\]MeNH\(_2\) and \[^{3}H\]IMP were obtained from the results of Fig. 6.

<table>
<thead>
<tr>
<th>Drug</th>
<th>[^{3}H]IMP</th>
<th>[^{14}C]MeNH(_2)</th>
<th>[^{3}H]IMP</th>
<th>[^{14}C]MeNH(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPZ</td>
<td>0.973</td>
<td>0.963</td>
<td>0.892</td>
<td>0.972</td>
</tr>
<tr>
<td>PPR</td>
<td>0.989</td>
<td>0.974</td>
<td>0.988</td>
<td>0.984</td>
</tr>
<tr>
<td>NH(_4)Cl</td>
<td>0.987</td>
<td>0.985</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 10. Concentration dependency of the intralysosomal accumulation of IMP.** The open circles represent the observed values of the concentration ratio (intralysosomal/extralysosomal) of IMP at various extralysosomal concentrations of IMP (at 4°C for 10 min). The dotted line was calculated using eq. 7, and the solid line was calculated using eq. 8. Buffer: 0.3 M sucrose, 2 mg/ml BSA, 20 mM HEPES-TMAH (pH 7.4).
[10,900 ± 4,700 and 62 ± 31 (mean ± S.D.), respectively]; the observed values for [14C]MeNH₂ were comparable to the expected values, whereas those of [3H]IMP were approximately 140-fold higher than the theoretical values. The concentration ratios of [3H]IMP at pH 5.0, 6.0, 7.0, 8.0, and 8.5 [315, 1,280, 5,360, 12,400, and 14,900, respectively (Fig. 4)] were also orders-of-magnitude larger than the theoretical values. The concentration ratios of the basic drugs at high-affinity/low-capacity sites (lysosomes) to the external buffer, estimated by subtracting the values at pH 5.0 from the total (assuming that the uptake at pH 5.0 reflects just binding at low-affinity sites), were also higher than the theoretical values and depended on the pH gradient [762, 3,790, 8,130, and 9,880 at pH 6.0, 7.0, 8.0, and 8.5, respectively (Ishizaki et al., 1996)]. These results suggest the operation of additional mechanism(s), other than pH partition, in the uptake of lipophilic bases in lysosomes.

These lipophilic basic drugs increased the intralysosomal pH (Fig. 5) and inhibited the uptake of [3H]IMP, as well as that of [14C]MeNH₂, at lower concentrations than did NH₄Cl (Fig. 6, Table 1). Also, the inhibitory effect of these lipophilic drugs on the uptake of [3H]IMP paralleled the potency to elevate intralysosomal pH and showed a good correlation with lipophilicity (Pₘoct). The additional mechanism(s), therefore, should correlate such activities with the lipophilicity of the bases. Among possible mechanisms are the pH-gradient-dependent uptake of bases accompanied by binding (partition or adsorption) of the protonated bases to lysosomal hydrophobic constituents (e.g., membrane or matrix lipidic polymers) and/or aggregation (dimerization or self-association) of the protonated bases. In this article, we derived three equations correlating the drug concentration and the intralysosomal pH: the first one is based only on pH-partition theory, the second is based on the same theory combined with a binding mechanism, and the last is based on the same theory combined with an aggregation mechanism (eqs. 1, 2, and 3, respectively, of Appendix). Using the β-value (buffering capacity of lysosomes) and the Kᵢ (binding constant) and K₂ (dimerization constant) values calculated for CPZ and PPR by applying these equations at the intralysosomal pH (Table 2), the inhibitory effects of these drugs on the uptake of [3H]IMP and [14C]MeNH₂ were simulated (Fig. 6). The simulated curves fitted well with the observed values. These results suggest that the inhibitory effects of NH₄Cl are due to the increase in intralysosomal pH, and the inhibitory effects of CPZ and PPR are also connected with the binding and/or aggregation of the protonated bases within lysosomes.

The elevation of intralysosomal pH by lipophilic bases, however, seems to be determined essentially on the basis of binding rather than aggregation because the concentration ratio of IMP is higher at lower concentrations (Fig. 9) and the simulated values obtained by assuming binding were close to the observed values below 1 μM. Namely, lipophilic basic drugs raise the intralysosomal pH at lower concentrations than do NH₄Cl because the protonated bases accumulate within lysosomes then bind (partition or adsorb) to the lipidic components of lysosomes (with or without aggregation), thus shifting the equilibrium to favor the entry of further lipophilic base molecules, which consume more protons and accumulate within lysosomes. This idea is consistent with the literature (Lüllmann and Wehling, 1979), which suggests that the interaction between a number of monovalent cationic amphiphilic drugs and several polar lipids can be considered as a partition of the drugs between a water phase and a dispersed lipid phase; the charges seem to play only a minor role. Adsorption on charged lipids in membranes (Gescher and Po, 1978; Desai et al., 1994) rather than partition of protonated bases, however, cannot be neglected. Also, participation of self-aggregation of protonated bases within lysosomes cannot be completely ruled out because there are lipophilic weak bases (phenothiazine drugs (Atherton and Barry, 1985) and local anesthetic drugs, tetracaine and propracaine (Mertz et al., 1990)) that tend to aggregate with critical micelle concentrations of millimolar order (comparable to the K₂ values for aggregation). Furthermore, there are some basic drugs (e.g., procaine, atropine, propranolol, and so on (Okhuma and Poole, 1981; Okhuma and Takano, 1997)) that cause osmotic vacuolation at relatively low concentrations, which is hard to explain on the basis of “binding.” The physical and chemical background of the findings (partition of ion pairs of protonated form (Cheng et al., 1990); equilibrium electric potential distribution (Ohshima et al., 1985); hydrophobic adsorption of charged molecules (McLaughlin and Harary, 1976); electrostatic interactions, and so on) will need to be clarified in future studies.

In conclusion, we showed in this article that lipophilic basic drugs are taken up by lysosomes via a ΔpH-driven mechanism, and higher lipophilicity increases the concentration ratio due to interaction of the drug with lysosomal lipidic compounds (membranes, etc.). This finding may be relevant to the frequently observed lipidosis or phospholipidosis induced by cationic amphiphilic drugs, most of which are actually present as hydrophobic protonated basic drugs (Halliwell, 1997). The findings described in this article should be applicable to interactions among lipophilic weak basic drugs in clinical therapy and, therefore, may help clinicians to avoid adverse effects of lipophilic basic drugs.

Appendix

Base Accumulation and Intralysosomal pH Based on Simple pH-Partition Theory. It is well accepted that the concentration ratio between intra- and extralysosomal space (F) of any basic drug depends on that of [H⁺] as described by eq. 9 (de Duve et al., 1974):

\[ F = \frac{C_{in}}{C_{out}} = \frac{K + [H^+]_{in}}{K + [H^+]_{out}} \]  

where \([H^+]_{in}, [H^+]_{out}\), and K represent intralysosomal and extralysosomal proton concentrations, and proton dissociation constant (Kₐ) of the protonated basic drug in question, respectively.

In this study, \(K \approx [H^+]_{in}\) and \([H^+]_{out}\) because the observed value of intralysosomal pH was 5.5 and the pH of the extralysosomal buffer was 7.4, whereas the K values of the basic drugs used in this study ranged from \(10^{-6.6}\) to \(10^{-10.4}\), except for the diprotonated basic drugs (Table 1). Under this condition, eq. 9 can be simplified to eq. 10:

\[ F = \frac{[H^+]_{in}}{[H^+]_{out}}, \text{ thus } C_{in} = \frac{[H^+]_{in}}{[H^+]_{out}} \cdot C_{out} \]
The buffering capacity (β) of lysosomes is defined as:
\[
\beta = \frac{C_{in}}{\Delta pH}, \text{ thus } C_{in} = \beta \cdot \Delta pH
\]
(11)
where ΔpH and \(C_{in}\) represent the pH difference and the amount of protons consumed (equal to the amount of protonated base accumulated) during the process of the pH change. From eqs. 10 and 11, eq. 12 is derived:
\[
\frac{[H^+]}{[H^-]}_{out} \cdot C_{out} = \beta \cdot \Delta pH, \text{ thus } C_{out} = \beta \cdot \Delta pH \cdot \frac{[H^+]}{[H^-]}_{in}
\]
(12)

For those basic drugs that are not bound or aggregated within lysosomes, the amount of protons consumed within lysosomes is regarded as equivalent to the amount of protonated bases accumulated within the lysosomes, and the relationship between the concentration of the extralysosomal basic drug \(x\) and intralysosomal pH \(y\) at medium pH 7.4 is described by eq. 1:
\[
x = \beta \cdot (y - N) \cdot \frac{10^{-7.4}}{10^{-y}}
\]
(1)
where \(N\) represents the original lysosomal pH (−5.5) in the absence of drug.

**Base Accumulation and Intralysosomal pH Based on pH-Partition Theory plus Intralysosomal Binding of the Base.** Assuming that the basic drug taken up within lysosomes is bound (partitioned or adsorbed) to the lipidic components (e.g., membrane), the total intralysosomal concentration of the basic drug \(C_{in,tot}\) is given by eq. 6:
\[
C_{in,tot} = [B]_{in} + [BH^+]_{in,t} + [BH^+]_{in,b}
\]
(13)
where \([BH^+]_{in,t}\) and \([BH^+]_{in,b}\) represent free and bound \([BH^+]\) in lysosomes, respectively. The \([BH^+]_{in,b}\) is expected to depend on \([BH^+]_{in,t}\), the amount of lipidic substances of lysosomes, and the partition (affinity) coefficient of the drugs for them, as given by eq. 14:
\[
[BH^+]_{in,b} = K_1 \cdot L \cdot [BH^+]_{in,t}
\]
(14)
where \(K_1\) and \(L\) represent the partition (affinity) coefficient and the amount of lysosomal lipidic substances (e.g., membranes), respectively. Then, \(C_{in,tot}\), the sum of the free and bound bases, is given by eq. 15:
\[
C_{in,tot} = C_{in,t} + K_1 \cdot L \cdot [BH^+]_{in,t}
\]
(15)
where \(C_{in,t}\) is the total intralysosomal concentration of the free (unbound) form of the basic drug. Therefore, the concentration ratio \(F = \frac{C_{in,tot}}{C_{out}}\) can be transformed to eq. 16 using eq. 15:
\[
F = \frac{C_{in,t} + K_1 \cdot L \cdot [BH^+]_{in,t}}{C_{out}}
\]
(16)
Considering the equilibrium, \([B] + [H^+] ⇔ [BH^+]\), and the equations \(K = \frac{[B][H^+]}{[BH^+]}\) and \(C_{in,t} = [B]_{in} + [BH^+]_{in,t}\), we obtain eq. 17:
\[
[BH^+]_{in,t} = \frac{[H^+]_{in}}{K + [H^+]_{in}} \cdot C_{in,t}
\]
(17)
Therefore,
\[
F = \left(1 + K_1 \cdot L \cdot \frac{[H^+]_{in}}{K + [H^+]_{in}}\right) \cdot \frac{C_{in,t}}{C_{out}}
\]
(18)
As \(K \ll [H^+]\), and \(\frac{C_{in,t}}{C_{out}} = \frac{[H^+]_{in}}{[H^+]_{out}}\) (eq. 10), eq. 7 is obtained from eq. 18:
\[
F = \left(1 + K_1 \cdot L \right) \cdot \frac{[H^+]_{in}}{[H^+]_{out}}
\]
(7)
As \(C_{in,tot} = F \cdot C_{out}\), eq. 19 is obtained from eq. 7:
\[
C_{in,tot} = \left(1 + K_1 \cdot L \right) \cdot \frac{10^{-7.4}}{10^{-y}} \cdot C_{out}
\]
(19)
As \(C_{in,tot} = \beta \cdot (y - N)\) (eq. 11), eq. 19 is transformed to eq. 20:
\[
C_{out} = \frac{\beta \cdot (y - N)}{1 + K_1 \cdot L} \cdot \frac{10^{-7.4}}{10^{-y}}
\]
(20)
If we take lysosomal membranes as the major lipidic substances within lysosomes, then \(L = k \cdot A\), where \(k\) and \(A\) represent the thickness and surface area of lysosomal membranes, respectively. The ratio of the surface area to the volume of lysosomes and the thickness of the membranes have been estimated to be \(1.1 \times 10^6 \text{ cm}^{-3}\) and 10 nm, respectively (de Duve et al., 1974). The volume of lysosomes and the amount of protein per tube in this study were \(3.27 \pm 0.17 \mu g/\text{mg}\) of protein (mean ± S.D., \(n = 5\)) and 65 to 80 \(\mu g\) (mean 72.5 \(\mu g\)), respectively. From these values, \(L\) is calculated to be 26.3 \(\mu g\). The relation between \(x\) and \(y\) is described by eq. 2:
\[
x = \beta \cdot (y - N) \cdot \frac{10^{-7.4}}{1 + K_1 \cdot L} \cdot \frac{10^{-y}}{10^{-7.4}}
\]
(2)

**Base Accumulation and Intralysosomal pH Based on pH-Partition Theory plus Intralysosomal Aggregation of the Base.** Assuming that basic drug taken up in lysosomes aggregates by producing the dimer, \(C_{in,tot}\) will be described by eq. 21:
\[
C_{in,tot} = [B]_{in} + [BH^+]_{in} + ([BH^+]_2)_{in}
\]
(21)
where \(([BH^+]_2)_{in}\) represents the concentration of the dimer within lysosomes. As the dimerization constant \(K_2\) is given by eq. 22:
\[
K_2 = \frac{[BH^+]_2}{[BH^+]_2^2}
\]
(22)
\(C_{in,tot}\) is given by eq. 23:
\[
C_{in,tot} = C_{in,t} + K_2 \cdot [BH^+]_{in}^2
\]
(23)
where \( C_{in,f} = [B]_{in} + [BH^+]_{in} \). Therefore, the concentration ratio \( F = \frac{C_{in,f}}{C_{out}} \) can be transformed to eq. 24 from eq. 23:

\[
F = \frac{C_{in,f} + K_2 \cdot [BH^+]_{in}}{C_{out}}
\]  
(24)

As \( K \ll [H^+] \), \([BH^+]_{in} = \frac{[H^+]_{in}}{[H^+]_{out}} \cdot C_{out} \) and \( C_{in,f} = \frac{[H^+]_{in}}{[H^+]_{out}} \cdot C_{out} \) (eq. 10). Therefore, eq. 24 can be transformed to eq. 8:

\[
F = \left( 1 + K_2 \cdot C_{out} \cdot \frac{[H^+]_{in}}{[H^+]_{out}} \right) \cdot \frac{[H^+]_{in}}{[H^+]_{out}} \cdot C_{out}
\]  
(8)

As \( C_{in, tot} = F \cdot C_{out} \), eq. 11 is transformed to eq. 25 using eq. 8:

\[
\beta \cdot (y - N) = \left( 1 - K_2 \cdot x \cdot \frac{10^{-y}}{10^-T} \right) \cdot \frac{10^{-y}}{10^-T} \cdot x
\]  
(26)

The relationship between \( x \) and \( y \) is given by eq. 27 (a quadratic equation):

\[
K_2 \cdot \left( \frac{10^{-y}}{10^-T} \right)^2 \cdot x^2 + \frac{10^{-y}}{10^-T} \cdot x - \beta \cdot (y - N) = 0
\]  
(27)

As \( x > 0 \), eq. 27 can be solved and \( x \) is given by eq. 3:

\[
x = \frac{\sqrt{1 + 4 \cdot K_2 \cdot \beta \cdot (y - N) - 1} - \frac{10^{-y}}{10^-T}}{2 \cdot K_2}
\]  
(3)

**Prediction of the Inhibitory Effect of a Basic Drug on the Uptake of Another Basic Drug into Lysosomes.**

Eq. 1 (describing the base accumulation based on simple pH-partition theory) is transformed to eq. 28 by replacing the intralysosomal pH \( (Y) \) with the increase of intralysosomal pH \( (Y = y - N) \):

\[
x = \frac{\beta \cdot Y}{10^{-T}}
\]  
(28)

When there is no interfering base (that is, \( y = N \)), the uptake amount \( (A) \) will be described by eq. 29:

\[
A = k_0 \cdot \frac{10^{-N}}{10^{-T}}
\]  
(29)

where \( k_0 \) represents just a proportional constant. The uptake amount \( (B) \) at arbitrary \( y \) value is given by eq. 30:

\[
B = k_0 \cdot \frac{10^{-y}}{10^{-T}}
\]  
(30)

When the intralysosomal pH is increased by the presence of another basic drug \( (x \ mM) \) and changes to \( y \), the ratio \( (Z) \) (between the presence and absence of the interfering basic drug) of the uptake of basic drug is described by eq. 31:

\[
Z = \frac{B}{A} = 10^{-(y - N)} = 10^{-y}
\]  
(31)

The relation between \( x \) and \( Z \) can be transformed to eq. 4:

\[
x = -\frac{\beta \cdot \log Z}{10^{-T}}
\]  
(4)

Similarly, eqs. 5 and 6 are obtained for binding and aggregating lipophilic bases from eqs. 2 and 3, respectively:

\[
x = -\frac{\beta \cdot \log Z \cdot (1 + K_1 \cdot L)}{2 \cdot K_2} \cdot \frac{10^{-y}}{10^{-T}}
\]  
(5)

\[
x = \frac{\sqrt{1 + 4 \cdot K_2 \cdot \beta \cdot (\log Z) - 1} - \frac{10^{-y}}{10^-T}}{2 \cdot K_2}
\]  
(6)

Using eqs. 4, 5, and 6, the inhibitory curves for the control, binding, and aggregation cases can be calculated, respectively.

In the derivation of the above equations, we have neglected the contribution of the neutral form of lipophilic weak bases for the sake of simplicity because 1) their concentrations are usually very small compared with those of the protonated bases, at least between pH 5.0 and 7.4, and 2) their concentration should be the same inside and outside of the lysosomal membranes and should not depend on ΔpH. We have also neglected the binding of protonated bases from outside the lysosomes, again for the sake of simplicity, because its contribution seems to be small as judged from the effects of NIG. An adsorption isotherm-type consideration might be more appropriate in deriving the equations for binding, but this approach was not adopted for the sake of simplicity and also to avoid any presumption about partition or adsorption.

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