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LYSOSOMOTROPIC BEHAVIOR OF ADRENERGIC ANTAGONISTS IN INTERACTIONS WITH HUMAN NEUTROPHILS*

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Abstract—Autonomic neurohormones affect the secretory activity of neutrophils by modulating release of lysosomal enzymes in response to immunologic stimuli. Autonomic agents are also weak bases which might modify cell function by accumulating in the acidic interior of the lysosome, in addition to their receptor-mediated activity. We examined the association of the beta-adrenergic antagonist [^{3}H]dihydroalprenolol with human neutrophils and lysosome and membrane fractions derived from neutrophils, and the subcellular distribution of the photoaffinity-labeled beta-adrenergic ligand [^{3}H]azidobenzylcarazolol after incubation with intact cells. Isolated neutrophil lysosomes accumulated significantly more [^{3}H]dihydroalprenolol than isolated membrane preparations. Decreasing the transmembrane pH gradient markedly reduced [^{3}H]dihydroalprenolol accumulation by intact cells or lysosomes but not by membranes. Since [^{3}H]dihydroalprenolol accumulated by intact cells remained rapidly exchangeable, the photoaffinity ligand [^{3}H]azidobenzylcarazolol was used to assess ligand distribution after association with whole cells. After cell disruption, 18.5 ± 1.3% of this ligand appeared in the lysosome fraction as compared to 2.2 ± 0.6% in the membrane fraction. The secretagogue phorbol myristate acetate caused release of the ligand as well as lysosomal enzymes from cells. These findings suggest that there is significant pH-dependent lysosomal accumulation of beta-antagonists. This lysosomotropic interaction may be important both as it affects the sequestration and redistribution of the drugs, and as it might in some circumstances affect host defense functions of the neutrophil.

The autonomic nervous system and the immune system interact on many levels. Neutrophils have adrenergic receptors [1, 2], and adrenergic agonists may affect neutrophil activation [3] via modulation of adenyl cyclase activity [4, 5]. It has been noted [6] that adrenergic ligands have, to varying degrees, the structure of membrane-permeant weak bases and, therefore, might be expected to accumulate within the acidic neutrophil lysosome. Furthermore, uptake of the labeled ligand [^{3}H]dihydroalprenolol (DHA) has been noted not to co-distribute exactly with plasma membrane markers in disrupted cells [7]. We have examined the interactions between a group of beta-adrenergic ligands and the human neutrophil lysosome. Our findings indicate that some beta-blockers can accumulate in the neutrophil lysosome—both in isolated organelles and as local concentration within the intact cell—and raise intralysosomal pH, and may under some circumstances become part of the releasable pool of lysosomal contents when cells are stimulated to degranulate. Therefore, these agents may affect cellular function by mechanisms independent of their blockade of beta-adrenergic receptors.

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MATERIALS AND METHODS

Reagents. [^{3}H]Dihydroalprenolol (DHA) and [^{3}H]azidobenzylcarazolol (pABC) were obtained from New England Nuclear, Boston, MA. DL-Propranolol, L-alprenolol, L-epinephrine, D- and L-isoproterenol, carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), 9-aminoacridine, and Ficoll were obtained from the Sigma Chemical Co., St. Louis, MO. Hypaque was from Winthrop Laboratories (New York, NY), Hanks' balanced salt solution (HBSS) from Grand Island Biologicals (Grand Island, NY), and phorbol myristate acetate (PMA) from LC Industries (Woburn, MA).

Cell preparation. Heparinized blood was obtained from healthy volunteers after informed consent. Neutrophils (PMNs) were isolated by Hypaque–Ficoll density gradient centrifugation and dextran sedimentation following the method of Boyum [8]. Residual erythrocytes were removed with three cycles of hypotonic saline lysis. For DHA experiments using intact cells, PMNs at 4 million/ml were then suspended in HBSS containing 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and 0.8 mM ascorbic acid, adjusted to pH 7.4. For 9-aminoacridine monitoring, cells were suspended in HBSS at 100 million/ml.

Cell fractionation. For experiments with cell components, neutrophils were suspended in HBSS with 2.5 mM $MgCl_2$ and no calcium (pH 7.5), disrupted by nitrogen cavitation at 350 psi for 20 min at 4°, then collected in EDTA to chelate the magnesium.

Differential centrifugation was then used to separate subcellular organelles into fractions containing undisrupted cells and nuclei (pellet 1), lysosomes (pellet 2), and plasma membrane vesicles (pellet 3). As previously described [9], this procedure yields approximately 70% recovery of lysosomes and 95% recovery of plasma membrane markers, with virtually no cross-contamination of these two fractions. Pellets 2 and 3 were then resuspended in HBSS/HEPES/ascorbic acid buffer and used for ligand binding studies.

Measurement of DHA association with cells and cell components. Suspensions of intact cells or pellets 2 and 3 as described above were divided into 1-ml aliquots. Each aliquot was incubated with 1–20 nM DHA at 37° for time periods ranging from 30 sec to 10 min. Incubation was terminated by adding 3 ml of ice-cold buffer and immediately filtering the sample through cellulose filters (5 micron pore) in a filtration manifold (Millipore Corp., Bedford, MA). These filters retained 84 ± 14% of plasma membrane vesicles and 95 ± 5% of lysosomes contained in the samples, as assessed by ^{125}I-labeling for membranes and lysozyme measurement for lysosomes. Filters were washed with 8 ml of cold buffer and placed in 10 ml of scintillation fluid (Aquasol, New England Nuclear). After dissolving overnight, samples were counted in an LS7000 liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA). Identically processed samples containing DHA but no cell components were used to determine background radioactivity adhering nonspecifically to the filter. These background counts were subtracted from sample counts.

Cell or cell-component-associated counts per minute (cpm) were expressed as femtomoles of DHA, using known quantities of DHA as standard counts. Results are reported as femtomoles DHA per 10 million cells or components derived from 10 million cells. For some experiments, protein assays were performed on whole cell preparations, lysosomes, and membranes by the method of Bradford [10] using globulin standards.

Volume determinations. In selected experiments, the volume of cells or lysosomes present was calculated from supernatant and pellet radioactivity after incubation with $^{3}H_2O$ and centrifugation through silicone oil as previously described [11]. This figure was corrected for medium trapped within the pellet by subtracting the pellet volume obtained after incubation of duplicate aliquots with [^{3}H]polyethylene glycol or [^{14}C]sucrose, to yield a net value for internal volume.

The ratio of internal to external concentration of DHA (C_i/C_o) was determined from the calculated volume and the pellet and supernatant radioactivity after incubation of duplicate lysosome aliquots with DHA. The ability of lysosomes and intact cells to concentrate DHA against a gradient was then compared.

Effect of other adrenergic ligands on DHA association. To assess competition by propranolol for DHA uptake, cell components were isolated from one neutrophil suspension and divided into two equal aliquots. Whole cells, lysosomes, and plasma membrane vesicles were resuspended in buffer with or without 0.1 mM propranolol, and DHA association was measured as described above, using a 10-min incubation.

Dissociation of DHA from intact PMNs was measured by incubating the cells with 1 nM DHA at 37° for 10 min, removing and filtering 1-ml aliquots to determine total cell-associated radioactivity, and adding propranolol (final concentration 0.1 mM) to the remaining cell suspension. Incubation at 37° was continued, and 1-ml aliquots were removed at intervals for measurement of remaining cell-associated radioactivity, which was expressed as a percentage of that measured before the addition of propranolol.

In other experiments, adrenergic agonists were used as competitors for DHA. L-Epinephrine was used at concentrations up to 1 mM. Both stereoisomers of isoproterenol were used to assess stereospecificity of competition, at concentrations up to 1 mM.

Effect of pH. In some experiments, cells and cell components were incubated for 10 min with DHA after resuspension in HBSS (with 0.8 mM ascorbic acid) pre-adjusted to pH 5, 7, or 9 with NaOH or HCl. The effect of the proton ionophore CCCP (1 or 50 μM) on DHA association with intact cells or cell components was also studied. Previous experiments [11] have established that cell viability remains over 92% at these pH values and in the presence of CCCP.

Temperature dependence. To assess whether DHA uptake was an energy-requiring process, the effect of temperature on whole cell association was measured. Uptake studies were performed as described above, but parallel incubations of split cell samples were carried out either at 37° or in an ice bath (0°).

Distribution of photoaffinity label. To investigate the subcellular fate of an adrenergic ligand incubated with intact cells, the photoaffinity reagent pABC was employed [12]. PMNs at 10 million/ml were incubated with 1 nM pABC in foil-wrapped tubes at 37° for 30 min in HBSS. Incubation was terminated by photolyzing the reaction mixture for 90 sec with longwave ultraviolet light (Blak-Ray B-100A lamp, Ultraviolet Products, San Gabriel, CA) to induce covalent binding of the pABC to sites of beta-adrenergic ligand penetration [13]. The photolyzed cells were fractionated as described above, and total radioactivity associated with each cell component was determined.

To assess release of pABC after association, the photolyzed cells were incubated with the secretagogue PMA for 20 min at 37°. Then release of radioactivity into the medium was measured in parallel with release of lysosomal enzymes (lysozyme, assayed [14] turbidimetrically as lysis of *Micrococcus lysodeikticus*, or beta-glucuronidase, measured [15] spectrophotometrically as release of phenolphthalein from its glucuronide), or postsecretory cells were fractionated as previously described. Lactate dehydrogenase [16] in the medium was used as a determinant of cell death.

Effect of adrenergic ligands on intralysosomal pH. The method for following intralysosomal pH in intact neutrophils has been described previously [17]. Briefly, fluorescence was monitored at 400 nm excitation and 456 nm emission in a solution (3 ml) of 0.05 μM 9-aminoacridine maintained at 37° by a cir-

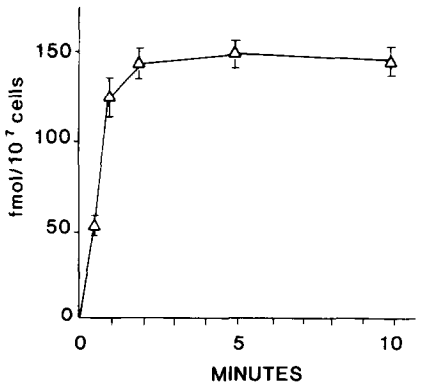

Fig. 1. Association of DHA (1 nM) with intact neutrophils (mean ± SEM of three experiments).

culating water bath. Neutrophils (120 μl at 100 million/ml for a final concentration of 4 million/ml) were added, and fluorescence quenching was followed for 4 min as the indicator was taken up into the lysosomes. Adrenergic agents were then added, and displacement of the indicator from the cells because of lysosomal alkalinization was monitored as increasing fluorescence. The degree of lysosomal alkalinization was estimated from the percent reversal of initial fluorescence quenching, as correlated with previous observations of the transmembrane pH gradient in isolated lysosomes [18].

Statistical treatment. Results are expressed as mean ± SEM of repeated experiments unless otherwise indicated. Student's *t*-test (two-tailed) was used for analysis of statistical significance.

RESULTS

Kinetics of DHA uptake. DHA association with intact neutrophils was rapid, reaching a maximum of 146 ± 11 fmol/10 million cells by 5 min of incubation with 1 nM DHA at 37°, with no additional accumulation by 10 min (Fig. 1). DHA association with isolated lysosome or membrane vesicle fractions was equally rapid, although membrane accumulation continued to rise slightly throughout the time period (Fig. 2). Lysosomes derived from 10 million cells took up a maximum of 5.5 ± 1.9 fmol DHA, whereas membrane vesicles took up 0.7 ± 0.1 fmol/10 million cells. Uptake by lysosomes was more than six times that by membrane vesicles from the same number of cells at all time points examined.

When uptake was expressed in terms of fmol DHA per mg of protein, lysosomal uptake of DHA was still greater than that by membrane vesicles. DHA association with lysosomes was 43.1 ± 4.7 fmol/mg protein compared with 21.8 ± 4.1 fmol/mg protein for membranes ($P < 0.01$ by *t*-test for unpaired samples with 22 df). Intact cells took up 85.2 ± 16.2 fmol DHA/mg protein.

On a volume basis, lysosomes accumulated DHA at least as avidly as intact PMNs. The calculated C_i/C_o for lysosomes after 10 min was 210 ± 66, compared with 182 ± 53 for intact PMNs. This comparison could not be made for membrane vesicles using these methods because the vesicles do not sediment through silicone oil.

Since equilibration of DHA with cells and cell components was rapid, further experiments were designed to reflect maximal uptake. The 10-min time period was used for all subsequent assays.

Association of DHA with intact PMNs was approximately linear over the concentration range of 1 to 10 nM (Fig. 3). No evidence of saturation was seen at values up to 20 nM (data not shown). Lysosome fractions also showed linear uptake at concentrations up to 10 nM (Fig. 4). Membrane vesicle preparations showed a nonlinear dose–response relationship with a decreasing slope, suggesting saturation of binding sites at high concentrations (Fig. 5).

Inhibition by competing ligands. Incubation with 0.1 mM propranolol produced profound inhibition of DHA uptake by intact cells, lysosome fractions, and membrane vesicles throughout the range of DHA concentrations tested (Figs. 3–5). Very high concentrations of agonists were required to inhibit uptake, and inhibition was only partial for whole cells and lysosomes. L-Epinephrine (1 mM) inhibited whole cell uptake by 19 ± 4%, lysosome uptake by

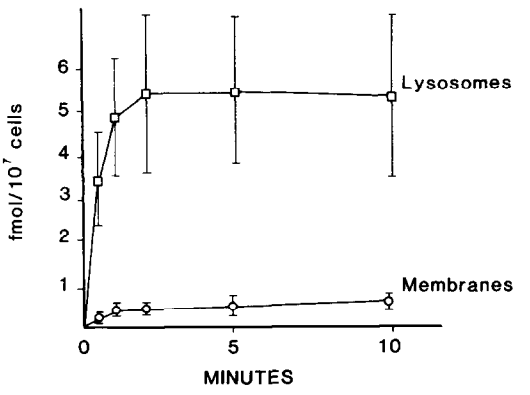

Fig. 2. Association of DHA (1 nM) with lysosomes and membranes, expressed as fmol DHA associating with lysosome or membrane fraction derived from 10 million cells (mean ± SEM of three experiments).

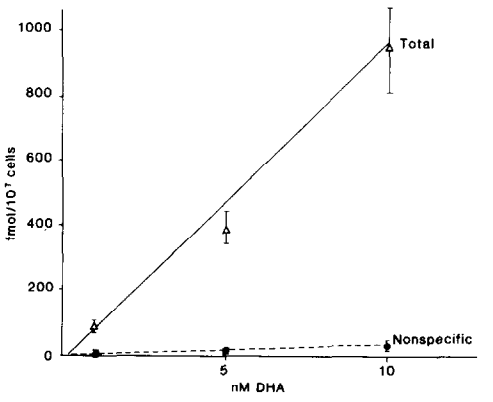

Fig. 3. Effect of DHA concentration on DHA association with intact neutrophils (mean ± SEM of nine experiments), and inhibition by 0.1 mM propranolol (two experiments). Here and in Fig. 4, lines represent regression lines derived from plotted points by the least squares method. Closed symbols labeled "nonspecific" represent DHA association in the presence of propranolol in Figs. 3–5.

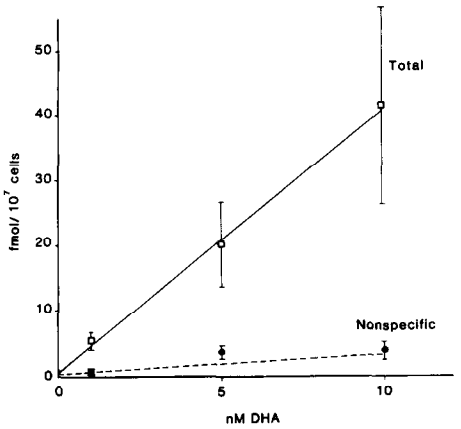

Fig. 4. Effect of DHA concentration on DHA association with lysosome fraction (seven experiments), and inhibition by 0.1 mM propranolol (closed symbols, mean of two experiments).

46 ± 8%, and membrane vesicle uptake by 89 ± 8%. Inhibition by 1 mM L-isoproterenol was 20 ± 4% in intact cells and 62 ± 17% in lysosomes, and no definite stereospecificity could be demonstrated.

Dissociation after uptake. Cell-associated DHA diminished rapidly after the addition of propranolol to the suspension (Fig. 6). Nearly 90% of cell-associated radioactivity was lost before the first time point measured (about 30 sec), and only 6% remained after 5 min. This suggests that DHA taken up by the cell remains freely exchangeable and re-equilibrates rapidly in the presence of a competitive inhibitor.

Effect of pH variation. The association of DHA with intact PMNs and cell components varied directly with the pH of the incubation media. At pH 5, uptake by intact PMNs was 13 ± 2% of control uptake measured at pH 7. Lysosomal uptake fell to 22 ± 3% of control, while membrane uptake was not altered significantly.

Alkaline medium increased DHA uptake. At pH 9, uptake by both intact PMNs and membrane vesicles approximately doubled: whole cell uptake was 217 ± 21% and membrane uptake 194 ± 49% of that observed at pH 7. Lysosome uptake rose to 134 ± 13% of control.

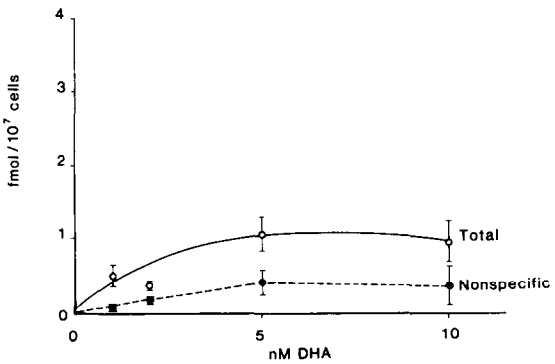

Fig. 5. Effect of DHA concentration on DHA association with plasma membrane fraction (six to thirteen experiments for each concentration), and inhibition by 0.1 mM propranolol (two to three experiments, closed symbols).

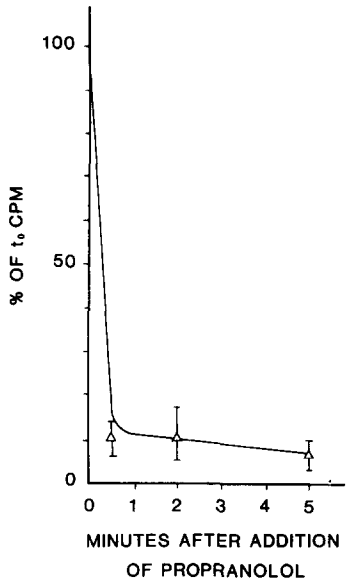

Fig. 6. Dissociation of DHA from intact neutrophils: percent of initial cell-associated cpm remaining at indicated times after addition of 0.1 mM propranolol (three experiments).

The use of carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) to facilitate equilibration of hydrogen ions across the membrane produced effects similar to those seen after incubation at acid pH. Following a 5-min preincubation with 50 μM CCCP, whole PMN accumulation of DHA fell from 94 ± 14 to 20 ± 3 fmol/10 million cells (N = 4; $P < 0.05$ by *t*-test for paired samples, 3 df). Lysosomal uptake fell from 5.0 ± 0.7 to 2.7 ± 0.4 fmol/10 million cells (N = 5; $P < 0.02$ by *t*-test for paired samples, 4 df). Membrane vesicle uptake showed a nonsignificant change from 1.1 ± 0.3 to 1.0 ± 0.3 fmol/10 million cells. When CCCP was used at 1 μM, PMN uptake was inhibited by 32 ± 12% and lysosomal accumulation by 40 ± 3%, while membrane binding was not reduced consistently.

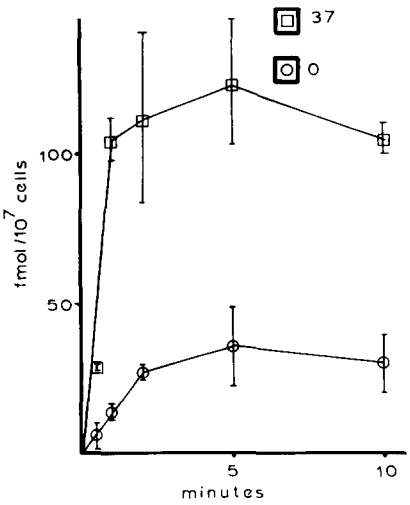

Fig. 7. Effect of temperature on DHA association with intact neutrophils (mean of two experiments).

Table 1. Release of lysosomal markers by PMA

	% Release of		
	Lysozyme	β-Glucuronidase	pABC
Unstimulated	9.3	0	4.5
PMA	29.6	4.2	7.9

Values are the mean of four experiments for pABC and the mean of two experiments for lysosomal enzymes.

Temperature effects. Whole cell uptake of DHA exhibited a marked temperature dependence (Fig. 7). At 0°, uptake at 10 min reached only 28% of the values attained at 37°.

Distribution of photoaffinity reagent. The total association of pABC with intact cells varied from day to day, but the cell component distribution relative to total uptake was consistent. Of total cell-bound pABC, 18.5 ± 1.3% could be identified in the lysosome pellet as compared to 2.2 ± 0.6% in the membrane fraction. Most of the remainder (40.5 ± 5.3%) was accounted for by the first pellet containing undisrupted cells and nuclei, whereas only 8.5 ± 1.2% appeared in the cytoplasmic fraction.

Cells incubated in the presence of 1 mM propranolol took up 49.8 ± 2.7% as much pABC as untreated cells. When propranolol-treated cells were fractionated, 19.2 ± 5.9% of total pABC was found in the lysosome fraction and 3.6 ± 1.4% in the membrane pellet.

Release of photoaffinity reagent. Table 1 shows the effect of the degranulating stimulus PMA on release of lysosomal enzymes and pABC into the medium. After ligand binding and photolysis, the secretagogue increased release of neutrophil-associated pABC into the medium by 80.4 ± 14.2% ($P < 0.05$ by single sample t-test with 3 df) relative to spontaneous release in the absence of PMA. The proportion of total cell-associated radioactivity in the lysosome fraction fell by 38.9 ± 8.2% after PMA. These differences were not attributable to increased cell death and disintegration after PMA treatment since lactate dehydrogenase release was actually less in PMA-stimulated cells (13% as opposed to 20%, mean of two experiments).

Lysosomal alkalinization by adrenergic ligands. Table 2 shows the effect of adrenergic ligands on intralysosomal pH as assessed by displacement of 9-aminoacridine with resulting release of fluorescence quenching. Both propranolol and alprenolol produced a dose-related rise in lysosomal pH. Propranolol at 1 mM also raised internal pH of isolated lysosomes, whereas epinephrine and isoproterenol at the highest concentrations practicable using this method (10–50 μM epinephrine, 10–250 μM isoproterenol, limited by solubility in stock solutions) had minimal or no effect on either intact cells or isolated lysosomes (data not shown).

Table 2. Lysosomal alkalinization by beta-blockers

Concn (mM)	% Reversal of quenching by Propranolol	Alprenolol
0.01	4.2	4.3
0.1	11.2	8.8
1	37.2	19.0

Values are mean of two experiments. From previous observations [18], 20% reversal corresponds approximately to a 0.5 pH unit rise and 30% reversal to a 1.5 unit rise in intralysosomal pH.

DISCUSSION

The neutrophil can be considered an end-organ of the autonomic nervous system in the sense that its functions are modulated by both adrenergic and cholinergic agonists via alterations in nucleotide cyclase activity. In this system of counterbalancing influences, beta-blockers inhibit binding of adrenergic agonists to receptors and the increases in cAMP leading to depression of leukocyte functions such as degranulation, but they have no intrinsic activity in stimulus–response coupling. However, many of the beta-blockers chemically are weak bases which should penetrate membranes in their uncharged form and accumulate in intracellular compartments such as the lysosome, and may thus directly affect cellular responses by means independent of their receptor binding. We have found that certain adrenergic antagonists do behave in this fashion.

Intact neutrophils took up DHA much more actively at 37° than at 0°, and uptake was inhibited far more efficiently by propranolol than by isoproterenol or epinephrine. While binding of DHA to plasma membrane vesicles was saturable and was inhibited profoundly by epinephrine, as would be anticipated for receptor–ligand association, binding to intact cells was not saturable over the concentration range under study. This combination of findings suggests that a major mechanism of uptake in the intact neutrophil is not governed by receptor binding. This is in accordance with the report of Dulis and Wilson [6] that neutrophil uptake of DHA can be inhibited by lysosomotropic bases which are not adrenergic ligands, and with the similar findings of Meier and Ruoho [19] in HeLa cells: propranolol should compete more effectively than epinephrine or isoproterenol for pH-dependent lysosomal accumulation because the greater charge of the agonists used should retard their diffusion across membranes into the lysosome.

If DHA is taken up by neutrophils along a pH gradient, several characteristics of its uptake should be predictable. First, accumulation should be greatest in acid intracellular compartments such as the lysosome. In accordance with this, we found that isolated lysosomes took up DHA far more avidly than plasma membrane vesicles and were able to concentrate the ligand against a large concentration gradient. The internal to external concentration ratio achieved was remarkably similar to that which would be predicted from the 2 pH unit gradient we have measured across the membrane of isolated lysosomes [18]. This gradient should approximate the log of the equilibrium distribution ratio of a permeant weak base [20]—that is, in this case, about 2.3. The pABC experiments also demonstrated intralysosomal

accumulation in the intact cell, which was further confirmed by the ability of the beta-blockers to displace 9-aminoacridine.

Second, DHA uptake should be inhibited by maneuvers which decrease the pH gradient across the lysosome membrane. This prediction was confirmed from two different approaches. Addition of CCCP to equilibrate hydrogen ions across the membrane should decrease the transmembrane pH gradient by redistributing the available protons, while experiments in acid medium should increase the gradient by adding hydrogen ions to the side with the lower concentration. We have demonstrated previously the diminished transmembrane pH gradient in these situations [18], and the present experiments showed diminished DHA uptake after both interventions.

Third, increasing transmembrane pH gradients should increase DHA uptake. The experiments performed in alkaline medium demonstrate this in several ways. Lysosomes, with a substantial pH gradient at external pH 7, showed modest enhancement of DHA uptake at pH 9. Membrane vesicles formed at physiologic pH should not have an acidic interior relative to the medium at external pH 7, so uptake was not inhibited at pH 5 because there was presumably no pH-dependent component to inhibit, but was enhanced when a pH gradient was created by raising external pH to 9. Intact neutrophils also showed very marked enhancement of uptake at pH 9, which could be explained on the assumption that the cytosol as well as the lysosome compartment was then acid relative to the medium.

Experiments with 9-aminoacridine confirmed that propranolol and alprenolol shared the behavior of lysosomotropic weak bases which, when they reach high enough concentrations in the lysosome, can buffer intralysosomal acid and raise the compartmental pH. The behavior of these agents was similar to that of clindamycin and chloroquine in our previous studies and showed a similar dose–response [21, 22]. An analogous effect of propranolol on pH gradients across a liposome membrane has been demonstrated [23]. The minimal effects of epinephrine and isoproterenol on intralysosomal pH can again be explained by their highly charged structure, which should impede transmembrane diffusion and intralysosomal accumulation.

The concentrations at which lysosomal alkalinization was detected were substantially higher than those achieved in serum after therapeutic doses of beta-blockers [24–26]. They might, however, be relevant to levels experienced by reticuloendothelial cells in the liver, which extracts up to 99% of propranolol from the circulation [27], or to situations in which these drugs are used topically [28].

Given this behavior of the various adrenergic agents, it would be predicted that weakly basic beta-antagonists may affect neutrophil function in a manner analogous to that observed for other lysosomotropic weak bases. We have reported previously that propranolol is among the bases that inhibit neutrophil degranulation in response to various soluble stimuli [17]. This agent has also been shown to inhibit the hexosemonophosphate shunt and nitroblue tetrazolium reduction [29] but not phagocytosis [30]; killing of yeast is not affected at micromolar concentrations [31], and chemotaxis is stimulated at low concentrations but inhibited by millimolar levels [29]. Inhibition of the hexosemonophosphate shunt and nitroblue tetrazolium reduction is compatible with our findings of dose-dependent inhibition of superoxide production by other lysosomotropic weak bases [22] and the resulting inference that alkalinization of intracellular acidic compartments by these agents may interfere with NADPH oxidase activation. Failure to inhibit phagocytosis is in accord with our observation that phagocytosis is inhibited by chloroquine but not by ammonium chloride and, therefore, is not dependent on pH maintenance in a strictly generalizable fashion [32].

The photoaffinity ligand studies confirm that adrenergic antagonists are lysosomotropic in the intact cell and suggest that these agents may subsequently be released when the neutrophil is stimulated to degranulate. Depolarization-mediated release of beta-blockers from synaptosomes has also been reported [33]. Therefore, intracellular sequestration and re-release may be a mechanism for tissue distribution and local activity of this class of drugs.

Thus, we have demonstrated that selected beta-adrenergic antagonists associate with the human neutrophil lysosome studied either as an isolated organelle or in the intact cell, and behave as would be predicted for lysosomotropic weak bases. These findings delineate an alternative modality of interaction between these drugs and the neutrophil which may function in parallel with beta-receptor-mediated events. In addition to the implications for *in vitro* dissection of cellular function, these relationships may in some settings contribute to the modulation of neutrophil activation.

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