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SELECTIVE LYSOSOMAL UPTAKE AND ACCUMULATION OF THE BETA-ADRENERGIC ANTAGONIST PROPRANOLOL IN CULTURED AND ISOLATED CELL SYSTEMS

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Abstract—The beta adrenoreceptor antagonist propranolol is rapidly taken up and accumulated in various cultured cell lines. When incubated in the presence of low concentrations of propranolol (10^{-9} M), Hela (non-differentiated epithelia), BC_3H_1 (smooth muscle) and MDCK (differentiated kidney epithelia) cell cultures take up ($t_{\frac{1}{2}}$ = 4–10 min) and accumulate the drug such that the intracellular concentration is over 1000 times that in the incubation medium. The release of propranolol from the cells was slower ($t_{\frac{1}{2}}$ = 22 min) than the rate of uptake but the dissociation was stimulated by the addition of 1 μM propranolol to the external medium ($t_{\frac{1}{2}}$ = 9 min). Uptake, which is non-stereoselective, is dependent on pH and is inhibited by the lysosomotropic agents, NH_4Cl, methylamine and chloroquine. At higher concentrations ($>10^6$ M), uptake is accompanied by a visual swelling of intracellular acidic vesicles staining with acridine orange. These results suggest that propranolol, a basic amphiphilic amine, is accumulated within the lysosomes of these cells. Uptake was confined to these cultured cell systems with no chloroquine-sensitive propranolol uptake, being found in isolated rabbit ventricular myocytes, red blood cells or blood platelets.

Although alprenolol and cyanopindolol competed with propranolol for uptake, isoprenaline, adrenaline, noradrenaline, phenylephrine, atenolol, practolol and salbutamol were not effective inhibitors.

The possible consequences of this uptake and accumulation of propranolol by certain tissues is discussed in relation to the known actions of the drug, particularly during or after abrupt withdrawal from chronic applications.

INTRODUCTION

Abrupt withdrawal of chronic propranolol therapy from patients suffering from coronary artery disease has been reported to result in the development of unstable angina or myocardial infarction [1–3]. These coronary events have been related to the transient increase in beta-adrenergic sensitivity which has been reported in normal subjects [4] or patients with hypertension [5] following periods of chronic propranolol administration and may possibily result from the increase in tissue beta-adrenergic receptor density found experimentally after prolonged propranolol treatment [6–8]. Although reports have indicated that there may be a general increase in tissue beta-adrenergic receptor density as a result of chronic propranolol therapy, it is not known if all tissues recover their normal receptor densities at the same rate following withdrawal of propranolol treatment. Indeed, we have previously shown that in the rat, withdrawal from chronic propranolol treatment results in differential rates of recovery of heart rate and blood pressure responses to i.v. isoprenaline [8]. The hypersensitive chronotropic response to i.v. isoprenaline found after withdrawal from propranolol treatment was transient in nature and was closely correlated with the changes in the beta-adrenergic receptor density/adenylate cyclase activity of the myocardium, whereas the enhanced blood pressure response was maintained for longer periods [8] indicating possible differential rates of recovery of myocardial and vascular smooth muscle from chronic beta-blockade.

Propranolol, a basic amphiphilic amine, is known to accumulate in the lung, liver, brain, kidney and intestine [9–12] as well as certain peripheral tissues [13], possibly as a result of lysosomal uptake [14, 15]. As high concentrations of propranolol are known to inhibit mitochondrial oxidative phosphorylation [16, 17] and various intracellular enzyme activities [17–19], tissue-specific uptake and release could result in substantial tissue variations in the extent and duration of both beta-adrenergic blockade and inhibition of cellular respiration, especially after abrupt withdrawal from prolonged treatment. Lysosomal uptake of propranolol and other lysosomotropic agents over prolonged periods may also alter tissue sensitivity to other agonists by disruption of the lysosomal involvement of the endocytotic pathways by which certain hormones mediate their cellular response [20, 21].

This paper reports the results of a preliminary study on the kinetics of propranolol uptake and release using various cultured or primary cell systems as models for tissue uptake *in vivo* in an attempt to investigate the possibility that selective lysosomal uptake of propranolol is involved with the hypersensitive states reported after abrupt withdrawal from drug therapy [4–6]. In particular the extent of propranolol uptake in isolated ventricular myocytes and cultured smooth muscle cells (BC_3H_1) were stud-

ied as models for drug accumulation in cardiac and vascular smooth muscle respectively. The methods employed were similar to those reported by Dulis and Wilson in the study of amine uptake by polymorphonuclear leukocytes [14]. Results from these experiments are discussed with respect to work previously carried out *in vivo* using the rat [8] and also to the clinical features associated with propranolol withdrawal in man [1–5]. A preliminary account of this work has previously been presented in abstract form [22].

MATERIALS AND METHODS

Cell culture. All cells were grown for 4 days at 37° in 120 cm glass bottles or 3 cm^2 plastic plates seeded at 3×10^4 cells/cm^2. All media used was equilibrated with 95% air/5% CO_2.

(a) BC_3H_1 smooth muscle cell line was a gift from Dr J. Patrick [23]. Cells were grown in Dulbecco's modified Eagles medium containing 10% (v/v) foetal bovine serum, 5 mM glutamine and 100 units/ml penicillin/streptomycin [24]. Confluent cultures yielded cells with volumes of 2041 ± 133 μm^3 and 1.64 ± 0.11 pl intracellular H_2O/cell.

(b) HeLa cells, obtained from Flow Laboratories (Irvine, U.K.), were grown in Eagles Basal Medium supplemented with 10% (v/v) new-born calf serum, 5 mM glutamine and 100 units penicillin/streptomycin [24]. Cell volumes were 2332 ± 84 μm^3 with 1.86 ± 0.20 pl intracellular H_2O/cell.

(c) MDCK dog kidney epithelial cells were obtained from Flow Laboratories (Irvine, U.K.) and were used at either 60–75 (Strain 1) or 110–120 (Strain 2) serial passages. These two cell lines have previously been shown to exhibit many different physiological and biochemical characteristics [25, 26]. Cells were grown in Eagles Minimum Essential Medium supplemented with non-essential amino acids, 10% (v/v) foetal bovine serum, 2 mM glutamine and 1 unit/ml kanamycin [24]. Cell volumes were 2384 ± 117 μm^3 with 1.92 ± 0.09 pl intracellular H_2O/cell.

Myocytes. Ventricular myocytes were prepared from 10- to 15-week-old, male New Zealand White rabbits by essentially the method reported for the rat [27]. Hearts from heparinized rabbits (7500 units i.v.) were rapidly removed into ice-cold Krebs–Henseleit bicarbonate buffer containing 118 mM NaCl, 14.5 mM $NaHCO_3$, 2.6 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$, 11.0 mM glucose maintained at pH 7.4 by gassing with 95% O_2/5% CO_2. Hearts were perfused as described previously [28] with the following modifications. Buffer volumes were: initial wash-out, 150 ml; recirculated perfusion medium, 200 ml; post-perfusion digest, 75 ml containing 0.2 mg trypsin and 0.2 mg DNAse I. Collagenase concentration was 0.3 mg/ml. Cell volumes of 18,290 ± 1062 μm^3 were recorded giving an intracellular H_2O content of 14.70 ± 0.88 pl/cell.

Red blood cells/blood platelets. Blood was removed from 14- to 18-week-old, male New Zealand White rabbits into 1/10 vol. 3.8% tri-sodium citrate, pH 7.4. Blood was cooled to 4° and centrifuged at 250 *g* for 30 min to yield an erythrocyte pellet and platelet-rich plasma. The buffy coat was removed from the pellet and erythrocytes were washed twice with 10 vol. modified Krebs solution containing 137 mM NaCl, 5.4 mM KCl, 1.2 mM $MgSO_4$, 0.3 mM NaH_2PO_4, 0.3 mM KH_2PO_4, 12 mM HCl, 14 mM Tris base and 11 mM glucose, pH 7.4 (KTG) before being resuspended to the original volume with the same buffer. Red cell volumes of 66 ± 2 μm^3 resulted in an intracellular H_2O space of 0.065 ± 0.001 pl/cell. Platelets were collected from the plasma by centrifugation at 1500 *g* for 30 min and resuspended in 3.8% tri-sodium citrate, pH 7.4. Platelet volumes were 7.5 ± 0.9 μm^3 with intracellular water space of 0.0036 ± 0.0007 pl/cell.

Cell numbers and mean volumes of cells in suspension (cultured monolayers were treated with KTG buffer containing 0.1% trypsin to yield single cell suspensions) were determined on a Coulter Counter (Model ZF) fitted with a Channelyser attachment (Model C1000). Myocyte volumes and numbers were calculated using suspensions containing 55–70% rod-shaped cells.

[^{3}H]*Propranolol uptake.* Cells grown on plates were incubated in 2.5 ml KTG containing tracer [^{3}H]-propranolol (2–4 nM, 0.04–0.08 μCi/ml) plus various concentrations of unlabeled propranolol or other effector. Cells were incubated at 37° for desired times before aspirating the medium and washing twice with 10 ml ice-cold KTG. Cells were removed from the plates by incubating in KTG containing 0.1% trypsin for 15 min at 37° and samples taken for assessment of cell numbers and associated radioactivity.

Cells in suspension were incubated in KTG buffer containing tracer [^{3}H]propranolol with or without effectors in 7.5 × 2.5 cm plastic incubation pots fitted with air-tight seals; in the case of myocytes these were gassed with 100% O_2 for 10–20 sec. at the start of the incubation and then at 30 min intervals. Pots were incubated at 37° in a reciprocal shaking water bath (100 cycles/min). Cell-associated propranolol was separated from free by either centrifugation of cells through oils (versilube; Alpha Chemicals or bromodecane/bromododecane; Aldrich Chemical Co.) [28, 29] or by rapid filtration on Whatmans GF/F filters followed by 2 × 5 ml washes with ice-cold KTG buffer.

[^{3}H]*Propranolol dissociation.* BC_3H_1 cells were preincubated with 4 nM [^{3}H]propranolol as outlined above. Plates were washed twice with ice-cold KTG buffer and release of associated propranolol from the cells was determined by infinite dilution. Plates were incubated in KTG containing 0.1% new-born calf serum (60 times original uptake buffer volume) and radioactivity and cell numbers determined at various times as above. Parallel experiments were carried out where dissociation was measured at 4° or in the presence of external unlabelled propranolol (1 μM).

Light microscopy. Cells were grown on coverslips in 3 cm^2 plastic plates for 3 days and then incubated for various times in normal growth media in the presence or absence of 1–100 μM propranolol or chloroquine. Coverslips were taken and incubated in KTG buffer containing 10 μg/ml acridine orange for 1 min at room temperature to stain intracellular acidic vesicles, washed in normal KTG buffer, and viewed under phase contrast or incident light fluore-

scent microscopy using a Leitz Dialux 20 Microscope fitted with a Ploemopak fluorescence illuminator. Acridine orange stained cells were viewed by fluorescence using an I2 filter block.

Materials. Tissue culture media sera and collagenase (type I) were obtained from Flow Laboratories, Irvine, U.K. Culture plates were obtained from Sterlin Ltd., Middx, U.K. (±)[^{3}H]Propranolol (20 Ci/mmole) was obtained from Amersham International. Unlabelled (+)-, (±)-propranolol, (±)atenolol and (±)practolol were obtained from ICI Pharmaceuticals, Macclesfield, Cheshire, U.K. and (−)isoprenaline, (−)adrenaline, (−)noradrenaline, (±)salbutamol and chloroquine were obtained from Sigma Chemical Co. Ltd. London. (±)Cyanopindolol was obtained from Sandoz, Feltham, Middx, U.K. Other general chemicals and reagents were of analytical grade and obtained from BDH, Dorset, U.K.

RESULTS

Propranolol at concentrations ranging from 10^{-9} to 10^{-4} M was rapidly taken up and accumulated by all cultured cell systems studied (BC_3H_1, HeLa, MDCK (strains 1 and 2)) with a steady state being reached after 40–60 min and half maximum uptake within 4–10 min (Fig. 1). No significant accumulation or association was found using cell homogenates (results not shown). The propranolol accumulated by the cells was not degraded even after prolonged incubations of up to 24 hr. Thin-layer chromatography on silica gel 60 plates (Merck) using butanol–water–acetic acid (12:5:3) showed that acid extracts of cell-associated [^{3}H]propranolol migrated identically to labeled or unlabeled propranolol (results not shown). Suspensions of red blood cells or blood platelets did not accumulate any propranolol over a 2-hr incubation period; however, incubations with ventricular myocytes resulted in an immediate but small uptake (Fig. 1).

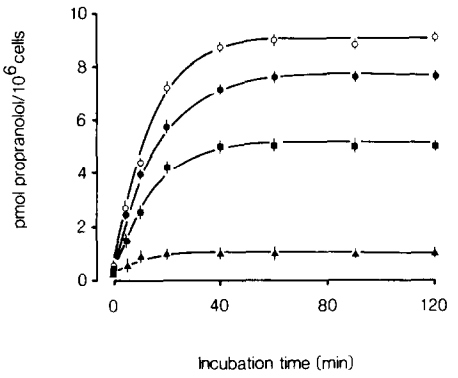

Fig. 1. Time course of propranolol uptake into HeLa (○—○), BC_3H_1 (●—●), MDCK (■—■) or ventricular myocytes (▲—▲). Cells were incubated at 37° in the presence of 4 nM [^{3}H]propranolol for the times indicated. Cells were isolated from the incubation medium (see methods) and uptake of radiolabel determined. Values are the means ± S.D. for three observations from a single representative experiment.

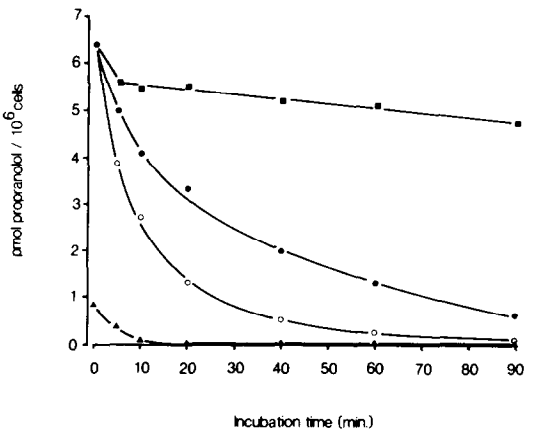

Fig. 2. Dissociation of propranolol from ventricular myocytes (▲) or BC_3H_1 cells (●, ○, ■). Cells were preincubated at 37° for 60 min in the presence of 4 nM [^{3}H]-propranolol, washed twice in ice-cold buffer and then incubated at 37° (●, ○, ▲) or 4° (■) in 60 vol. (BC_3H_1) or 10 vol. (myocytes) of fresh medium containing 0.1% bovine serum albumin. Dissociation of radiolabel from the cells was determined in the absence (●—●) or presence of 1 μM propranolol (○—○). Values are means from duplicate observations from representative experiments.

The rate of propranolol dissociation ($t_{\frac{1}{2}}$ = 22 min) from the BC_3H_1 muscle cell line was slower than the rate of uptake with 10% of the propranolol left associated with the cells after 90 min incubation (Fig. 2). The inclusion of propranolol in the incu-

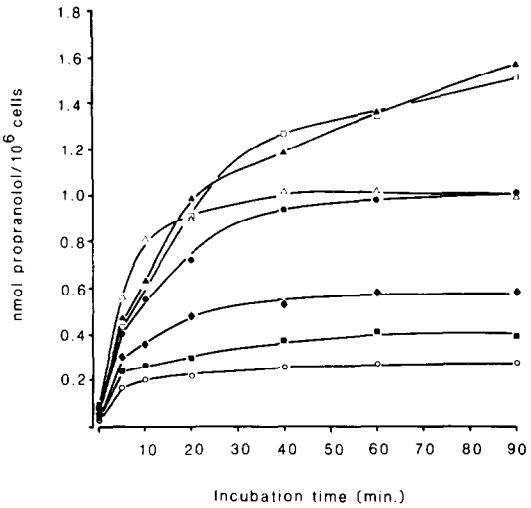

Fig. 3. Effect of extracellular pH on propranolol uptake into cultured BC_3H_1 cells. Cells were incubated at 37° in the presence of 4 nM [^{3}H]propranolol plus 1 μM unlabelled propranolol for the times indicated. The incubation medium was buffered to pH 6.0 (○—○), 6.5 (■—■), 7.0 (◆—◆), 7.4 (●—●), 7.8 (□—□), 8.2 (▲—▲) or 8.6 (△—△). Uptake of radiolabel was determined as in Methods. Values are means from duplicate observations from a representative experiment. Uptake of propranolol from the other cultured cell lines was also similarly dependent on extracellular pH.

bation buffer increased the dissociation rate with the $t_{\frac{1}{2}}$ being reduced to 9 min. Apart from an initial small reduction in associated propranolol during the first 5 min of incubation, decreasing the incubation temperature to 4° greatly inhibited release of the drug from the cells with 74% of the cell associated radioactivity still bound after 90 min.

Propranolol uptake into cultured cells, but not ventricular myocytes, was highly dependent on the medium pH with more alkaline buffers (up to pH 8.2) inducing an increase in both the rate of uptake and the total associated propranolol at the steady state (Fig. 3). More alkaline buffers increased the initial rate of propranolol uptake but reduced the steady-state accumulated level. When cells were incubated in the presence of therapeutic concentrations of the drug (10^{-9}–10^{-7} M), propranolol was accumulated such that the calculated intracellular concentration (assuming the drug was evenly distributed throughout the intracellular water space) was over 1000 times that of the incubation buffer. Uptake into cultured cells, but not ventricular myocytes, was inhibited by the lysosomotropic agents NH_4Cl, methylamine and chloroquine (Fig. 4), indicating lysosomal involvement. Incubation of cultured cells with 1–100 μM propranolol or chloroquine for periods up to 24 hr resulted in increases in lysosome number and volume as viewed under phase contrast or fluorescence microscopy (Fig. 5), again indicating a lysosomal involvement in drug uptake. Changes in the wavelength of fluorescent light emitted from the lysosomes of cells stained with acridine orange, which accumulates in acidic vesicles, also indicated that an increase in lysosomal pH also accompanied swelling. No increase in lysosomal volume was found with myocytes under identical incubation conditions.

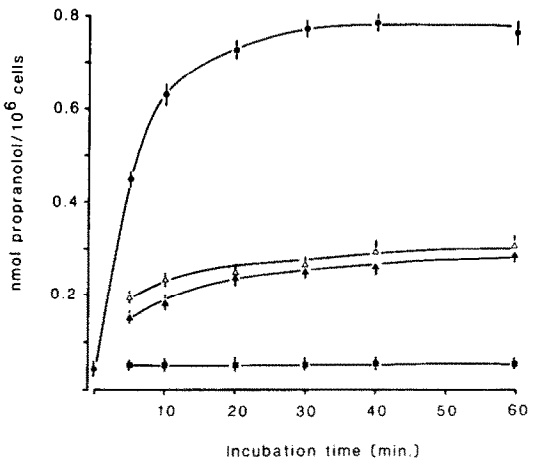

Fig. 4. Effect of chloroquine, methylamine and NH_4Cl on propranolol uptake into BC_3H_1 cells. Cells were incubated at 37° in the presence of 4 nM [^{3}H]propranolol plus 1 μM unlabeled propranolol in the absence (●—●) or presence of 100 μM chloroquine (■—■), 10 mM methylamine (△—△) or 10 mM NH_4Cl (▲—▲) at pH 7.4. Uptake was measured as given in Methods. Values are means ± S.D. for three observations from a single representative experiment. The other cultured cell lines gave similar responses.

Scatchard plots [30] of propranolol uptake into cultured cells yielded biphasic plots indicating the presence of both high affinity low capacity and low affinity high capacity sites. Analysis of the binding data for ventricular myocytes resulted in a linear plot indicating a single low affinity site (Fig. 6). Dissociation and maximum binding constants calculated for propranolol uptake into various cell types are shown in Table 1. All cultured cells show similarities in both high and low affinity association sites; however, isolated ventricular myocytes appear to have only low affinity binding sites for propranolol (Table 1) which are not sensitive to NH_4Cl or methylamine (results not shown). No significant uptake of [^{3}H]propranolol was found with either red blood cells or blood platelets.

Binding of propranolol to beta-receptors of adrenergic-sensitive cells (myocyte, BC_3H_1 and Strain 1 MDCK cells) was not detected due to the low concentration of receptors and the relatively low specific activity of the tritiated propranolol.

Of the beta-adrenergic agonists and antagonists investigated only alprenolol and cyanopindolol showed any marked inhibition of uptake with isoprenaline, adrenaline, noradrenaline, phenylephrine, atenolol, practolol and salbutamol having only minor effects (Table 2). Accumulation was not stereo-specific with (+)propranolol exhibiting similar uptake constants to that of (±)propranolol.

DISCUSSION

Propranolol has been shown to be rapidly accumulated by all cultured cell lines investigated, including BC_3H_1 (smooth muscle), HeLa (non-differentiated epithelia) and MDCK (differentiated kidney epithelia) cell systems. Uptake, which was independent of the catecholamine sensitivity of the cell (HeLa and MDCK type 2 cells are devoid of beta-adrenergic receptors), was half-maximal after 4–10 min and reached a steady state within 40–60 min. Propranolol does not appear to be degraded during this incubation period. As lowered extracellular pH and incubations in the presence of various lysosomotropic agents such as chloroquine, NH_4Cl and methylamine inhibit propranolol uptake, the lysosomes appear to be at least one possible intracellular storage site. The increases in the apparent number and volume of intracellular acidic organelles seen under the light microscope also indicates that the lysosomes are responsible for the majority of the cell accumulation. Other workers, using alveolar [31] or peritoneal [32] macrophages as well as polymorphonuclear leukocytes [14], have also indicated that lysosomal storage may account for uptake of propranolol as well as other amphiphilic amines. Uptake is considered to be a passive process where the permeable free base can diffuse rapidly across both the plasma and lysosomal membrane and as a result of the reduced pH the base is protonated, forming an impermeant cation, resulting in intra-lysosomal accumulation. The extent of drug accumulation would therefore depend on the pH differential between the external, cytosolic and the intra-lysosomal environments, the pK_a for protonation

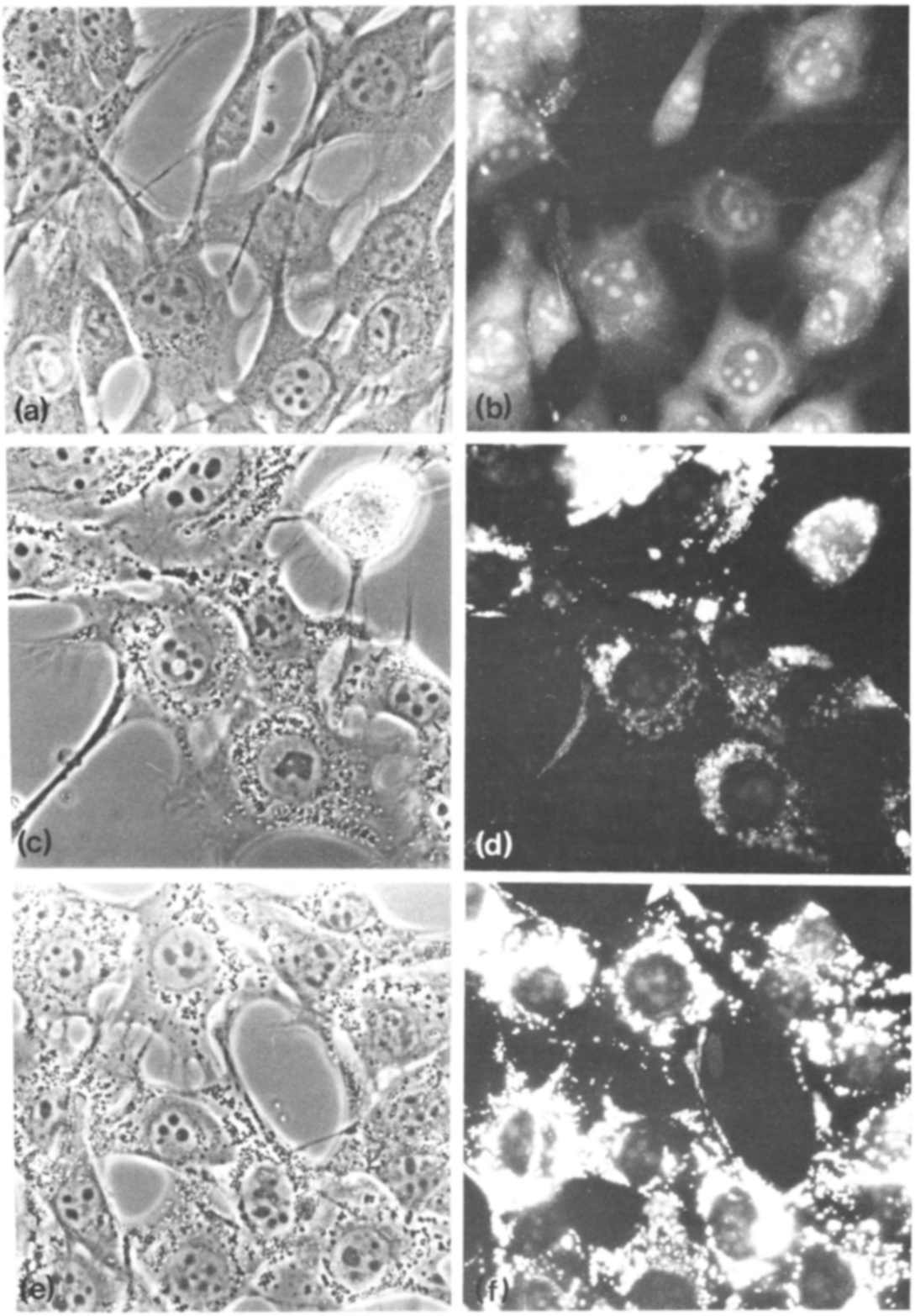

Fig. 5. Effect of propranolol and chloroquine on lysosomal swelling in cultured BC_3H_1 cells. Cells were incubated at 37° for 90 min in the absence (a, b) or presence of 50 μM propranolol (c, d) or 10 μM chloroquine (e, f). Cells were observed under phase (a, c, e) and fluorescence (b, d, f) microscopy. Magnification ×2100. Lysosomal swelling was also found with HeLa and MDCK cell lines.

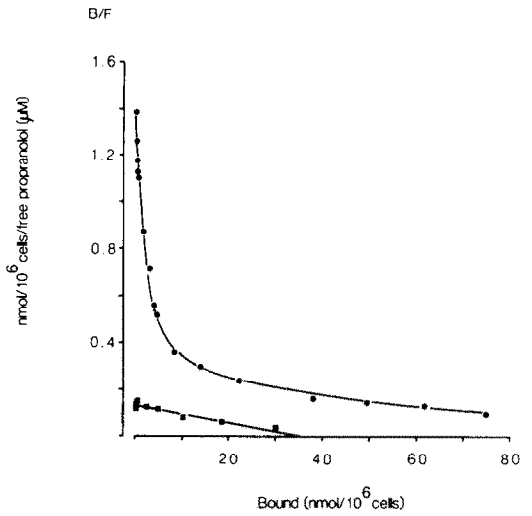

Fig. 6. Scatchard plot of propranolol uptake into BC_3H_1 cells (●) or ventricular myocytes (■). Cells were incubated at 37° for 60 min in the presence of 2 nM [^{3}H]propranolol plus increasing concentrations of unlabeled propranolol. Cell associated radioactivity was determined as in Methods. Ordinate: nmol propranolol per 10^6 cells/free propranolol concentration (μM). Abscissa: nmol propranolol per 10^6 cells. Values are means from three observations from one representative experiment.

Table 2. Inhibition of propranolol uptake into BC_3H_1 cells.

Beta-agonist/antagonist	Control uptake (%)
(±) Propranolol	29.4 ± 0.6
(+) Propranolol	32.1 ± 0.8
(−) Isoprenaline	86.9 ± 5.1
(−) Adrenaline	88.9 ± 2.0
(−) Noradrenaline	88.9 ± 2.9
(±) Phenylephrine	91.7 ± 3.7
(±) Atenolol	91.2 ± 2.3
(±) Salbutamol	93.4 ± 3.5
(±) Practolol	92.6 ± 3.2
(±) Alprenolol	52.8 ± 2.0
(±) Cyanopindolol	68.5 ± 1.5

BC_3H_1 cells were incubated at 37° for 60 min in the presence of 1 μM propranolol (plus 2 nM [^{3}H]propranolol). Beta-agonists or antagonists were added to the incubations at 25 μM and uptake of radiolabel assessed as in Methods. Values are means ± S.D. from three observations. Control uptake was 0.65 ± 0.19 nmol propranolol/10^6 cells.

and the relative membrane solubilities of the acidic and basic forms of the drug.

Incubation of cells in the presence of nanomolar concentrations of propranolol results in accumulation such that the calculated intracellular concentration of the drug throughout the total water space of the cell is over 1000 times that in the external medium. Similar investigations on amine uptake into polymorphonuclear leukocytes have indicated that a theoretical maximum concentration difference of 500-fold is possible between the external medium and the intra-lysosomal space [14]. Since the lysosomal volume was estimated to be around 1% of the cell volume there is clearly a large difference between the actual amount of propranolol accumulated by the cultured cells and that expected from theoretical calculations [14]. Assuming that the lysosomes are the sole sites of propranolol accumulation and since there are no indications of increases in lysosome number or volume at these low concentrations, the accumulation of the drug from external environment to intra-lysosomal environment must be considerably greater. Assuming the intra-lysosomal pH to be even as low as 4 (general intra-lysosomal pH is regarded to be around 4.5–5 [33–35]), the distribution of propranolol (pK_a 9.4) based entirely on the increased ionized form of the drug in an acidic impermeable compartment would require the lysosomal volume in the case of HeLa and BC_3H_1 cells to be nearly 50% and MDCK cells 26% that of the entire cell. The extent of the accumulation is clearly much greater than that expected due to trapping of the drug in the lysosomes solely on the basis of the decreased pH alone. The difference in uptake and release rates and the stimulated release in the presence of extracellular drug also indicate that other, at present unknown, factors are involved in propranolol accumulation.

Incubation of cultured cells in the presence of micro-molar concentrations of propranolol or chloroquine results in extensive accumulation of these amphiphilic agents within the lysosomes which in turn induces an increase in pH accompanied by osmotic swelling of the acidic vesicles. The fluorescent micrographs indicate that chloroquine appears to be more potent than propranolol at inducing lysosomal swelling. Chloroquine at concentrations of

Table 1. Dissociation and maximum uptake constants calculated from Scatchard analysis of propranolol uptake into cells

Cell	High affinity site K_d (μM)	High affinity site B_{max} (nmole/10^6 cells)	Low affinity site K_d (μM)	Low affinity site B_{max} (nmole/10^6 cells)
BC_3H_1	2.65 ± 0.22	2.65 ± 0.16	294 ± 44	94.5 ± 8.4
HELA	1.34 ± 0.12	4.33 ± 0.25	301 ± 50	101.0 ± 9.1
MDCK (Strain 1)	3.43 ± 0.21	2.77 ± 0.13	180 ± 30	61.7 ± 4.8
MDCK (Strain 2)	1.94 ± 0.25	1.93 ± 0.17	540 ± 90	100.3 ± 11.8
Ventricular myocyte	—	—	254 ± 39	34.7 ± 4.1

Values (means ± S.E.M.) were calculated by a computer-fitted linear regression of high and low affinity sites separated by Scatchard analysis of the data (Fig. 6).

1 μM induced lysosomal swelling approximately equivalent to that seen with 100 μM propranolol. One possible explanation for the reduced lysosomal swelling with propranolol compared to chloroquine is back-diffusion of the charged species through the lysosomal membrane into the cytosol [32, 35]. However, due to the increase in pH found in the cytosol the propranolol would be free to diffuse down its concentration gradient and out of the cell. Another explanation for the reduced vacuolation found with propranolol is that the hydrophobic ring structure at one end of the molecule is embedded in the inner half of the lysosomal membrane with the charged aliphatic side chain extending into the acidic milieu of the lysosome. Indeed, cell killing by lysosomotrophic detergents (basic amines with 9–14 carbon aliphatic tails) [36] may also result from a partial solubility in the lysosome membrane. Assuming a constant pH, such association of a proportion of the accumulated propranolol with the lysosome membrane would explain both the large uptake of propranolol by the cells and the higher concentrations of propranolol required to induce equivalent swelling to chloroquine.

The non-stereoselective uptake of propranolol suggests that the general chemical nature of the species is an important factor, especially in relation to lipid solubility, and uptake is unlikely to depend on a specific enzyme involvement. Propranolol accumulation in rat brain synaptosomes has also been shown to be non-stereo-selective [37], again indicating that uptake of the drug into acid vesicles is not related to receptor binding. The inability of the other beta-agonists and antagonists, with the exception of alprenolol and cyanopindolol, to inhibit uptake probably lies with the decreased lipid solubility of the uncharged species, making them impermeable to plasma and/or lysosomal membranes.

Although red blood cells which are devoid of lysosomes do not accumulate propranolol, lysosomal uptake and storage also appears to be tissue-specific since ventricular myocytes and blood platelets, which have been shown to contain acidic vesicles containing cathepsin D and other hydrolytic enzymes [38, 39], do not exhibit NH_4Cl or methylamine-sensitive propranolol uptake. This is in direct contrast to morphological studies where propranolol has been suggested to stabilize myocardial lysosomes [40]. This may not be a direct action, however, but may be due to a decreased work demand of the myocardium. In the case of ventricular muscle access of propranolol to the intracellular environment may be restricted by the highly polar nature of the glycocalyx of the sarcolemmal membrane [41]. The reduction in the medium pH which has been reported to surround negatively charged surfaces [42] such as the sarcolemmal glycocalyx would also contribute to propranolol impermeability. Carazolol, a related lipophilic beta-adrenergic antagonist, has also been reported not to accumulate in heart cells [43]. Whole body uptake studies also indicate that although propranolol is accumulated by certain tissues including the brain and liver, cardiac ventricular muscle does not concentrate the drug [11]. The inability of blood platelets to accumulate propranolol is, however, unknown.

Uptake of propranolol from the circulation decreases after prolonged drug treatment, indicating a tissue saturability [10]. After withdrawal from chronic drug therapy the circulating drug concentrations would fall due to the rapid plasma clearance [8, 44]. This would disturb the equilibrium between the circulating and accumulated drug resulting in release which may create extra-cellular microenvironments of relatively high propranolol concentration. Tissues which are sensitive to beta-adrenergic agonists and which accumulate propranolol may experience a more extensive and prolonged blockade than sensitive tissues which do not accumulate the drug. The resulting differences in beta-adrenergic sensitivity between certain tissues may be responsible for certain side-effects of the drug, particularly during withdrawal from chronic treatment [45]. As high concentrations of propranolol inhibit mitochondrial oxidative phosphorylation [16, 17], tissues which accumulate the drug may also experience reduced respiration rates.

As lysosomes are known to be involved in protein, phospholipid and low-density lipoprotein degradation [46–49], accumulation of propranolol and other lysosomotropic agents may possibly alter protein and lipid turnover rates within the cell. Propranolol has also been reported to inhibit certain enzyme activities associated with lysosomal function [18, 50] and as lysosomes and pre-lysosomal vesicles are involved in the turnover of certain plasma membrane proteins the beta-antagonist may inhibit receptor-mediated endocytosis [20, 21, 46]. It has previously been shown that, in the rat, there is a transient hypersensitive heart rate and blood pressure response to i.v. isoprenaline after abrupt withdrawal from chronic propranolol treatment [8]. Although the increased sensitivity of the heart rate response to isoprenaline returns to normal within 48 hr, the blood pressure response still shows an increased sensitivity to the beta-agonist indicating possible differential rates in tissue recovery from prolonged blockade. One possible explanation is that chronic propranolol treatment results in a general increase in beta-receptor density of all sensitive tissues and the rate of removal of these additional receptors is reduced in vascular smooth muscle, but not ventricular muscle, due to selective propranolol uptake and inhibition of lysosomal degradative processes.

These experiments therefore suggest that chronic treatment with propranolol may result in differential tissue distributions resulting from tissue-selective lysosomal uptake and accumulation. Such tissue distributions may contribute to the pharmokinetic and pharmodynamic properties of the drugs when given alone or in combination with other lysosomotropic agents. Also, as a result of the extensive accumulation and concentration of the drugs within certain tissues, the pharmacology of drug action may differ between tissues, particularly with regard to the rates of protein and phospholipid turnover. Selective tissue uptake and accumulation may result in differential tissue sensitivity to catecholamines and may contribute to some of the clinical features observed in the weeks following propranolol withdrawal.

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