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Uptake of Ibuprofen, Indomethacin and Ketoprofen into Isolated Rabbit Parietal Cells

MICHAEL J. DENINGER AND RONALD D. SCHOENWALD

Division of Pharmaceutics, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52245, USA

Abstract

In the United States and other countries, non-steroidal anti-inflammatory drugs (NSAIDs) can be purchased without a prescription. Due to their widespread use, the drugs' most common side effect, gastric toxicity, becomes a more serious concern. Gastric toxicity can occur directly by contact with mucous membranes or indirectly by the inhibition of prostaglandin production in the gastric mucosa. We have studied the uptake of NSAIDs in gastric tissue, specifically parietal cells removed from the rabbit stomach.

New Zealand White rabbits were killed and then used to harvest parietal cells. The purified cells were used to study the uptake of ibuprofen, indomethacin and ketoprofen (NSAIDs) over time and under different experimental conditions.

The effects of concentration were investigated for all three NSAIDs. In addition, indomethacin and ibuprofen were used to investigate the mechanism of uptake. Studies were determined for the effects of varied extracellular pH from pH 6 to 8, and inhibitory conditions from depressed temperature (5°C), metabolic inhibitors (sodium azide and 2,4-dinitro-phenol), an ionophore (nigericin) and a sodium free support medium. The interaction of NSAIDs with lysed parietal cells was investigated also.

Initial rate data indicated that Michaelis-Menten kinetics were apparent; however, poor solubility of all three NSAIDs prevented complete characterization of the inclusion of a passive transport mechanism. Uptake showed a statistically significant increase ($P = 0{\cdot}01$ to 0·05) as pH decreased, also suggesting contribution from a passive mechanism. Studies with inhibitors showed minimal effects. However, uptake at equilibrium for the ionophore, nigericin, showed a 10-fold increase over the control for ibuprofen ($P = 0{\cdot}005$) and a 1·4-fold increase for indomethacin ($P = 0{\cdot}04$). Depressed temperature (5°C) increased the initial rate and uptake at equilibrium 2·1- and 2·2-fold, respectively, for ibuprofen ($P < 0{\cdot}01$). For indomethacin depressed temperature increased the initial rate and uptake at equilibrium 2·7- and 5·2-fold, respectively ($P < 0{\cdot}01$). The increases at 5°C suggests that adsorption may be an important uptake component. Experiments with lysed parietal cells showed a non-specific uptake phenomenon, suggesting an adsorption component was occurring also.

With the development of new non-steroidal anti-inflammatory drugs (NSAIDs) as well as the assignment of many of these agents to non-prescription status, their use has increased. Their increased use in the general population, often without clinical supervision, has meant that the drugs' most common side effect, gastric toxicity, has become a more serious concern. The gastric toxicity of NSAIDs range from simple upset stomach to severe ulceration and perforation of the gastric mucosa. It has been estimated that NSAIDs are responsible for as much as 30% of all serious ulcer complications (Somerville et al 1986). Their gastric toxicity can occur directly by contact with mucous membranes or indirectly by inhibition of prostaglandin production in the gastric mucosa. The use of pharmaceutical methods, such as buffered dosage forms, enteric coating, sustained release formulations or prodrug formation have proven only moderately useful (Gengos 1978;

Correspondence: R. D. Schoenwald, Division of Pharmaceutics, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52245, USA.
E-Mail: rschoenw@blue.weeg.uiowa.edu

Rhymer 1979; Robinson 1983; Rainsford 1984; Brune 1985; Pease et al 1985; Lussier & LeBel 1987).

Cultured human intestinal epithelial (CACO-2) cell monolayers have been used extensively as a model for gastrointestinal transport and metabolism (Inui et al 1992; Chikale & Borchardt 1994; Tsuji et al 1994); however, for the study of uptake of selected NSAIDs, the acid-producing parietal cells were chosen as a model. Since the biodistribution of NSAIDs occurs to a significant degree in acidic areas of the stomach, kidneys, and areas of local lactic acidosis triggered by induced inflammation (Brune & Graf 1978; Brune et al 1984), the parietal cell was considered a more logical choice. Also, Rainsford (1975), Rainsford & Brune (1978) and McCormack & Brune (1987) speculated that the accumulation of drug activity could be in parietal cells. They observed that parietal cells were specifically damaged within a few minutes following the oral administration of aspirin, which was also supported by electron microscopy studies. It was hypothesized that parietal cells could act as a reservoir for NSAIDs because of their acidic nature.

If the mechanism of interaction between NSAIDs and parietal cells could be elucidated, the possibility exists that future drug design could benefit from this information so that the drug's affinity for parietal cells could be reduced. A method for isolating and purifying parietal cells from the stomach of the rabbit (Berglindh & Öbrink 1976; Berglindh 1985) has been developed and hence rabbit parietal cells were used in this study. The purpose of this study was to determine the extent of uptake of selected NSAIDs, ibuprofen, indomethacin and ketoprofen, into isolated rabbit parietal cells.

Materials and Methods

Chemicals

Indomethacin, ibuprofen and ketoprofen were used as received from Sigma Chemical Co. (St Louis, MO). [^{14}C]Indomethacin (30 mCi mmol^{-1}) was obtained from DuPont New England Nuclear (Wilmington, DE). [^{14}C]Ibuprofen (50 μCi mmol^{-1}) and [^{3}H-general]ketoprofen (4 Ci mmol^{-1}) were obtained from American Radiolabel Chemical (St Louis, MO). All radioactive NSAIDs were dissolved in various concentrations of ethanol and reported to be greater than 99% radiochemically pure, and therefore used as received. [^{3}H]Water was purchased from Amersham International (Buckinghamshire, UK) and used as received. Other general use chemicals were also used as received.

Preparation of gastric gland suspension from rabbit stomach mucosa

Male New Zealand White rabbits (B & F Farms, Newton, IA) were housed in the Animal Care Unit (The University of Iowa, Iowa City, IA). Rabbit raw gastric glands were isolated using a modification of the method outlined by Berglindh & Öbrink (1976). A rabbit was anaesthetized with an intramuscular injection of 1 mL of a mixture of ketamine 72 mg mL^{-1} (Aveco Co, Fort Dodge, IA), xylazine 11·7 mg mL^{-1} (Aveco Co), and acepromazine 1·7 mg mL^{-1} (Mobay Corp., Shawnee, KS). The rabbit was then killed by an intravenous injection of 0·5 mL 390 mg mL^{-1} pentobarbital sodium and 50 mg mL^{-1} phenytoin sodium (Beuthanasia-D Special, Schering-Plough Animal Health, Kenilworth, NJ) into the marginal ear vein. An incision was made on the abdomen just below the sternum, and the abdomen was opened to expose the stomach and intestines. The stomach was quickly removed by dissection starting at the greater omentum on the greater curvature of the stomach. An incision was made around the oesophagus and pylorus followed by dissection of the smaller omentum along the lesser curvature.

The dissected stomach was then rinsed with a warm solution of phosphate buffer (pH 7·4). After draining blood from the veins of the stomach, the stomach was opened with an incision from the oesophagus toward the non-glandular portion of the mucous tunica. The stomach contents were emptied and the incision lengthened towards the pylorus. The stomach was then inverted, rinsed with warm oxygenated phosphate buffer, and residual stomach contents removed by blotting the mucosa with filter paper. The cardial and antral regions were removed and discarded. The mucosa was then removed from the muscular and submucosal layers with a razor blade using a gentle backward scraping motion. The resulting gastric glands were then incubated with a 2% Pronase solution (Boehringer-Mannheim Corp., Indianapolis, IN) at 37°C for 15 min, then washed with respiratory medium and incubated with collagenase solution for 30 min (see Table 1). After incubation, the cell suspension was filtered through a 70 μm nylon mesh (Spectra/Mesh Nylon, Spectrum, Houston, TX) and centrifuged at 50 *g* for 5 min, followed by rinsing and suspension in warm oxygenated respiratory medium.

The gastric gland suspension was further processed to enrich the fraction of parietal cells. The cell suspension was carefully layered on top of a

Table 1. Ingredients of 2% Pronase and 2% collagenase solutions.

Ingredients	Collagenase (pH 7·4) (mg L^{-1})	Pronase (pH 7·4) (mg L^{-1})
NaCl	7·6	7·6
$NaHCO_3$	1·0	1·0
NaH_2PO_4	0·414	0·414
$Na_2HPO_4 \cdot 7H_2O$	0·804	0·804
K_2HPO_4	0·523	0·523
$MgSO_4 \cdot 7H_2O$	0·493	0·493
$CaCl_2$	0·147	0·147
Collagenase[a]	1·0	–
Pronase[b]	–	2·0
D-Glucose	2·0	2·0

[a]Sigma Chemical Co., St Louis, MO; added just before use.
[b]Boerhringer-Mannheim Corp., Indianapolis, IN; added just before use.

discontinuous gradient of Nycodenz (Sigma Chemical Co., density layers: 1·05 and 1·08 g L^{-1}). The three-layered sample was then centrifuged (Model CS, International Equipment Co., Needham Hts, MA) at 200 *g* for 10 min. Enriched parietal cells were carefully removed from between the 1·05 and 1·08 g L^{-1} interfaces with a Pasteur pipette, rinsed with at least 2 vols respiratory medium and centrifuged at 50 *g* for 5 min. The resultant pellet of enriched parietal cells was then re-suspended and rinsed three times in respiratory medium or modified respiratory medium (according to treatments i.e. modified pH, addition of metabolite inhibitors or sodium free composition). Cell purity was determined by counting the cells under a light microscope using a haemocytometer (Sigma Chemical Co.), as well as transmission electron microscopy (R. Nessler, The University of Iowa Electron Microscopy Research Facility). Cell viability was determined by colour changes using phenol red and from the use of the trypan blue dye exclusion test.

Procedure for cellular uptake of NSAIDs

Isolated, purified rabbit parietal cells in suspension were placed in a 25-mL flask, charged with oxygen and allowed to equilibrate for 15 min with gentle shaking. After equilibration, radiolabelled drug solution was added at a specific activity of approximately 25 μCi $mmol^{-1}$ to the cell suspension.

At time intervals of 1, 2, 3, 4, and 15 min, cells were separated from the drug solution and the reaction quenched by use of a modified silicone sandwich technique (Kazatchkine et al 1979). Briefly, 200-μL sample solution was removed using an Eppendorf repeating pipette and carefully introduced to a 400-μL microfuge tube (Fisher Scientific, Fair Lawn, NJ) previously filled with 100 μL of a 4 : 1 mixture of dibutyl phthalate (Fisher Scientific) and dinonyl phthalate (Fisher Scientific). The sample was then centrifuged for 45 s at 25 000 rev min^{-1}. The samples were then frozen in a dry ice/methanol bath. The tip of the microfuge tube containing the cell pellet was collected by carefully cutting the tube with a microfuge tube cutter. Care was taken to minimize the amount of organic solvents collected with the cells. The cell pellet was dissolved in 0·5 mL 1 M NaOH in a 7-mL glass scintillation vial for 1–2 h until all the cells were digested, and then neutralized by the addition of 0·5 mL 1 M HCl. After storing overnight to reduce chemiluminescence, the samples were mixed with scintillation fluid (Budget Solve, Research Products International, Mt Prospect, IL), and counted (Model 3801, Beckman Instruments, Fullerton, CA). Samples were corrected for background counts and converted to concentration using the specific activity associated with each sample. Counting efficiency was determined by an internal standardization technique (Ward & Schirmer 1977) (H number, Beckman Instruments). A separate portion of each sample was used to assay for protein content (Bradford 1976) so that uptake results were reported as pmol drug (μg protein)$^{-1}$.

Time course investigations were conducted at 37°C and pH 7·4. Cell samples were withdrawn (0·2 μL) at 1, 2, 3, 4, 5, 7·5, 10, 12·5, and 15 min after incubation and separated from the bulk medium by centrifugation at 25 000 rev min^{-1} for 45 s. After separation from the bulk fluid, the cell pellet was digested and assayed for radiolabelled drug and protein.

Drug concentrations ranging from 0·25 to 2·67 mM were studied for uptake into parietal cells at 37°C at 1, 2, 3, 4, 5, 7·5, 10, 12·5 and 15 min. Cells were removed from the bulk medium and treated as described above.

To assess the effects of extracellular pH on uptake, cell suspensions were adjusted in pH to 6·0, 6·4, 6·7, 7·4 (control), or 8·0 using either 0·1 M HCl or 0·1 M NaOH. For each of the various treatments described below, cell samples were incubated with NSAIDs, samples were withdrawn (0·2 mL) and then separated from the bulk medium at 1, 2, 3, 4 and 15 min and treated as described above.

To examine the role of an inwardly directed proton gradient across the cell membrane during uptake, the proton gradient was dissipated before measuring uptake by pre-incubation of the cell suspension in nigericin (Sigma Chemical Co.). Experiments were conducted at 37°C. Nigericin 10 μg mL^{-1} was pre-incubated 15 min before initiating uptake by the addition of drug.

Energy dependence of the uptake of NSAIDs was studied with the use of the metabolic inhibitors, 2,4-dinitro-phenol and sodium azide. Experiments with the metabolic inhibitors were conducted at 37°C. Concentrations of 0·5 mM 2,4 dinitro-phenol or 10 mM sodium azide were dissolved in respiratory medium and incubated for 15 min with parietal cells before the addition of the drugs.

To examine the role of reduced temperature on the uptake of NSAIDs, the cells and drug solutions were cooled separately in an ice-water bath maintained at 5°C. After temperature equilibration, the uptake experiments were initiated by mixing the drug and cell suspension.

The role of sodium ion on the uptake of NSAIDs into parietal cells was studied by substituting choline chloride (Sigma Chemical Co.) for NaCl in the respiratory medium. Some sodium ion was nevertheless present from the sodium phosphate buffer salts. However, a 92% reduction in sodium ion concentration, from 143 to 11 mM, was achieved with this substitution. Experiments were conducted at 37°C.

Determination of protein content

Although an approximate cell count was conducted for many of the samples, it was more appropriate to determine the protein content in each sample. The protein content in the samples was modified from the method of Bradford (1976) using the Bio-Rad protein assay kit (Bio-Rad Melville, NY). A 50-μl sample of the dissolved cell solution was placed in a 3-mL disposable polystyrene UV cuvette. Each sample was then mixed with 2·5 mL protein assay solution. After 5 min the samples were analysed for UV absorbance at 595 nm (Model UV 2100U, Shimadzu Corp., Kyoto, Japan). Rabbit serum albumin (fraction V, Sigma Chemical Co.) in concentrations ranging from 80 to 1600 $\mu g\,mL^{-1}$ was used as a standard to express initial rate of uptake or uptake at equilibrium as pmol (μg protein)$^{-1}$ min^{-1} or pmol (μg protein)$^{-1}$, respectively.

Determination of radiolabel stability and metabolism of NSAIDs by parietal cells

An HPLC system (solvent delivery system model LC-6A, Shimadzu Corp.) equipped with a radioisotope detector (model 171, Beckman Instruments) was used to determine that the ^{14}C and particularly the general label, ^{3}H, was intact and the radioactivity that was measured after uptake was associated with each of the NSAIDs. Samples of either the drug solution (before incubation) or the digested cells (after incubation) were injected onto a reverse phase 5 μm C-18 column (Axxi-Chrom 15 cm × 4·5 mm i.d., Waters Chromatography Div., Milford, MA) protected by a C-18 precolumn cartridge (μBondapak Guard-Pak, Waters Chromatography Div.). A syringe loading sample injector (model 7125, Reodyne Corp., Cotati, CA) was used along with a 100-μL loop (Reodyne Corp.) or an automated injector (model SIL-9A, Shimadzu Corp.). A solvent flow rate of 1·5 mL min^{-1} was used for all assays. The eluent from the column was mixed with scintillation fluid (Budget-Solve, Research Products International) at a flow rate of 3 mL min^{-1}. The mixture of scintillation fluid and eluent from the HPLC column was passed through the flow cell (600 μL, Beckman Instruments) of the radioisotope detector. The output was recorded on a printer (Epson LX-80 Dot matrix printer, Beckman Instruments). The radioisotope detector was calibrated before analysing the samples using a calibration cell (part number 523927, Beckman Instruments) containing the appropriate unquenched standards (Beckman Instruments). Standardization of tritium disintegrations min^{-1} was performed using standard tritiated water.

Investigation of surface binding

A known number (approximately 20 million) of purified parietal cells were resuspended with respiratory medium and then homogenized with a high shear mixer (Polytron, Brinkman Instrument Co., Switzerland) for 5 min (set at 8). Solutions of ibuprofen or indomethacin were prepared in respiratory medium and added to the cell homogenate to produce 6-mL suspensions containing concentrations of 0·33, 1·3, 2·0 or 2·7 mM. The suspensions were prepared to determine if adsorption to exterior and/or interior surface structures of parietal cells could explain the uptake phenomena. After 15-min incubation, 1 mL was removed and placed in a concentrator (Centicon-3, Amicon, Danvers, MA) and centrifuged (Centra-7r, International Equipment Co., Needham Hts, MA) at 7000 *g* for 2·5 h to separate the cell lysate from the supernatant solution. The cell lysate was then assayed for radiolabelled drug and for protein content.

Results and Discussion

Purity of the parietal content in the cell preparation was determined by cell count and found to be between 90–95% (mean ± s.d. = 92 ± 2%, n = 106

determinations). This result corresponded well to other published research (Berglindh & Öbrink 1976; Berglindh 1985; Mazzeo et al 1988; Ainz et al 1993). The remaining cells were mucous cells and chief cells, which were visible using electron microscopy. Using the trypan blue dye exclusion technique, viability was measured usually at about 95% through 90 min by cell count of parietal cells in suspension at 37°C without the presence of drug. Unless the cell viability was greater than 90%, the experiment was not considered valid. In preliminary experiments the viability of the parietal cells was also determined in the presence of naproxen (Sigma Chemical Co.) at relatively high concentrations of 13 and 65 μg mL^{-1} for 20 and 60 min incubation time, respectively. The results showed that at 20 min and 13 μg mL^{-1}, the viability of the cells was identical to the control (no drug present). At 60 min and 65 μg mL^{-1}, cell viability was reduced considerably below 90%. Since no experiment went beyond 15 min, cell viability was assumed to be satisfactory. The amount of water adhering to cells was determined with the use of tritiated water. Cells were incubated with and without the addition of tritiated water for about 30 s. The increase in radioactivity was negligible and could not be differentiated from background counts. Therefore no correction for water adherence was made.

Uptake into parietal cells

Time course for uptake. Figure 1 shows the average of three experiments summarizing the uptake of ibuprofen (2·5 mM), indomethacin (2·7 mM) or ketoprofen (2·7 mM) into parietal cells over time. The viability of the cells for each NSAID was greater than 95% for the 16-min time interval as measured by the trypan blue dye exclusion test. Initial uptake was very rapid, particularly for indomethacin and ketoprofen, reaching 75% of equilibrium within 2 min and complete equilibrium at approximately 4 min. Ibuprofen was slower, reaching 75% saturation at approximately 4–5 min and complete saturation at 8–16 min. Due to the rapid uptake, it was not logistically possible to include a number of measurements to approximate the linear initial rate. Therefore, two measurements were made, the initial rate calculated from the 0–1-min time interval and the saturated uptake measured from the last two time intervals, 12·5 and 15 min.

Concentration dependence. Uptake of ibuprofen or indomethacin into isolated, purified parietal cells was determined at four concentrations ranging from

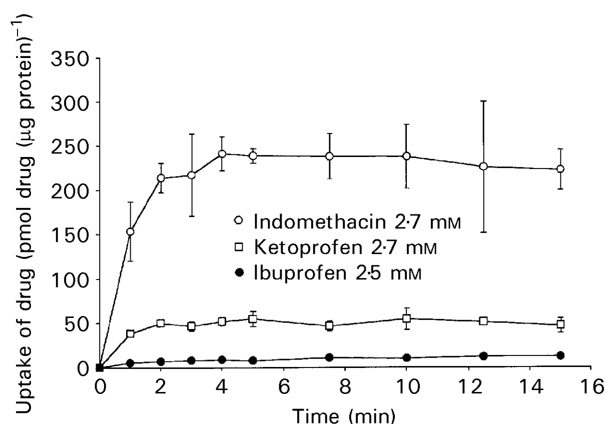

Figure 1. Time course of indomethacin, ketoprofen and ibuprofen uptake into isolated parietal cells removed from rabbit gastric mucosa. Data shown are mean ± s.d. of three to four determinations (pH 7·4) at each time interval.

0·25 or 0·5 mM to 2·5 mM and at a pH of 7·4. Ketoprofen was studied over a concentration range of 0·7–2·7 mM. Higher concentrations could not be used because of the limiting solubility of each NSAID which have been reported below 10 and 15 mM at pH 7·0 and 7·5, respectively (Herzfeldt & Kümmel 1983). A further complication arose from the possibility that, although the bulk pH was 7·4, the pH in the microenvironment could be somewhat lower because of acid secretion by the parietal cells. Figure 2A shows the relationship between uptake and the corresponding concentrations. Concentrations beyond 2·5 or 2·7 mM were not possible, therefore the exact plateaus for all three NSAIDs were uncertain. Thus, it was not possible to determine if a nonsaturable transport component was operating. Since it was not possible to eliminate the possibility of a nonsaturable component, the plateau could not be estimated and therefore both V_{max} and K_m for each NSAID could not be calculated with any confidence. Figure 2B is a plot of equilibrium uptake vs concentration, which appears somewhat linear for each NSAID, arguing for a passive component to uptake. Equilibrium concentrations (pmol drug (μg protein)$^{-1}$) from highest to lowest followed the rank order indomethacin > ketoprofen > ibuprofen.

Effect of pH on uptake. Uptake was investigated at pH values of 6·0, 6·4, 6·7, 7·4 and 8·0 for ibuprofen and indomethacin. Table 2 represents the initial uptake at each pH value. The pK_a values of ibuprofen and indomethacin are 5·3 and 4·6 (Herzfeldt & Kümmel 1983), respectively, therefore more of the drug was un-ionized at the lower pH values. For example, the percent un-ionized for ibuprofen and indomethacin ranged from 16·7 to 0·2% and from

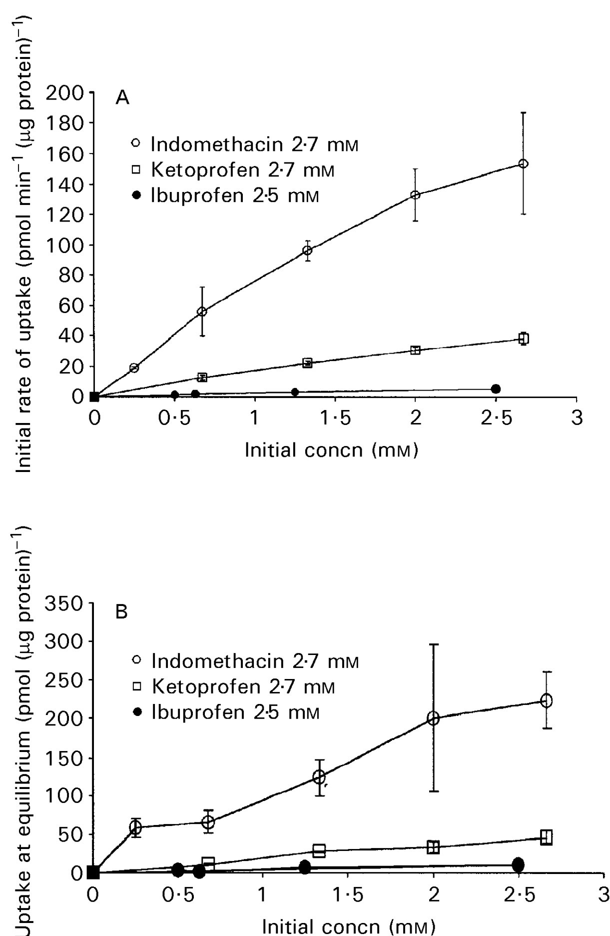

Figure 2. A. Concentration dependence of the initial rate of uptake for indomethacin, ketoprofen and ibuprofen. Each concentration is the mean ± s.d. of three to four determinations (pH 7·4) estimated between 0–1 min. B. Concentration dependence of uptake at equilibrium for indomethacin, ketoprofen and ibuprofen. Each concentration is the mean ± s.d. of three to four determinations (pH 7·4) estimated between 0–1 min.

3·8 to 0·04%, respectively, for pH values from 6 to 8. The increase in initial rate corresponded well with the rise of the percent un-ionized at low pH values, suggesting a passive transport component. The initial rates at pH 6 and 6·4 were both statistically significant from the control, pH 7·4 for ibuprofen, but only pH 6 was statistically different for indomethacin. If transport was occurring significantly via passive diffusion, indomethacin would not be expected to show a significant difference in uptake at pH 6·5 compared with pH 7·4 because of the lower pK_a for indomethacin; only a few percent of the drug is un-ionized at pH 6·5.

A small, statistically insignificant, increase in the initial rate was observed at pH 8·0. Table 2 shows also the equilibrium data, which yielded the same results for both ibuprofen and indomethacin. The effect of pH on the uptake of ibuprofen into parietal cells appeared to be associated with passive diffusion and partitioning. If an active transport mechanism was operating, it may have a hydrogen ion dependent component.

Table 2. Comparison of pH effects on the initial rate of uptake as well as uptake at equilibrium for ibuprofen and indomethacin.

Comparison	pH dependence	
	Ibuprofen	Indomethacin
Initial rate (pmol min^{-1} (μg protein)$^{-1}$)		
pH 6·0 vs 7·4	+++	+++
pH 6·4 vs 7·4	+++	0
pH 6·7 vs 7·4	−	0
pH 8·0 vs 7·4	++	+
Uptake at equilibrium (pmol (μg protein)$^{-1}$)		
pH 6·0 vs 7·4	+++	++
pH 6·4 vs 7·4	+++	0
pH 6·7 vs 7·4	0	0
pH 8·0 vs 7·4	++	++

0 no change; + or − small, non-significant change ($P = 0·1$); ++/−− a trend ($P = 0·05$ to 0·1); +++/−−− a significant change ($P = 0·05$ to 0·01).

Effect of various inhibitory conditions, 2,4-dinitrophenol, sodium azide, nigericin, reduced temperature and sodium dependence. Table 3 summarizes the effects of various inhibitory conditions on the initial rate of uptake into parietal cells. For both ibuprofen and indomethacin, the inhibitors 2,4-dinitro-phenol, sodium azide and nigericin showed no statistically significant effect on the initial rate compared with the control, although sodium azide exhibited a weak trend ($P = 0·06$) towards a decrease for ibuprofen. The experiments containing choline chloride substituted for sodium chloride (i.e. sodium free) showed a small but significant decrease ($P < 0·05$) suggesting a sodium dependent mechanism of uptake for ibuprofen but not for indomethacin.

The most surprising result was the highly significant ($P < 0·005$) increase in uptake observed for reduced temperature (5°C) for ibuprofen and indomethacin and for initial as well as equilibrium data. A cellular explanation entails an active transport mechanism for drug leaving the cell. However, at 5°C this is highly unlikely. A pharmaceutics explanation would be that the increase in drug uptake was not due to a transport mechanism but to a physical phenomenon such as adsorption of the drug to components of the cell. Adsorption phenomena, saturable and nonspecific, are thermodynamically more favourable at lower temperatures and could follow a similar mathematical interpretation as active transport.

Table 3. Comparison of the effects of several inhibitors on the initial rate of uptake and the uptake at equilibrium of ibuprofen and indomethacin. Statistical comparisons (* $P < 0.05$, ** $P < 0.01$) were made for each inhibitory condition (n = 6 for pH 7·4, 37°C control, and for 5°C; n = 3 for other conditions).

Inhibitor effects			Comments
Result	Ibuprofen	Indomethacin	
Initial rate (pmol min^{-1} (μg protein)$^{-1}$)			
5°C	++++	++++	Compared with 37°C, pH 7·4
2,4-Dinitro-phenol	0	0	0·5 mM
Sodium azide	0	0	10 mM
Nigericin	0	+ +	10 μg mL^{-1}
Sodium free	−−−	0	Choline chloride substituted for NaCl
Uptake at equilibrium (pmol min^{-1} (μg protein)$^{-1}$)			
5°C	++++	++++	Compared with 37°C, pH 7·4
2,4-Dinitro-phenol	−−−	0	0·5 mM
Sodium azide	0	+	10 mM
Nigericin	++++	+ + +	10 μg mL^{-1}
Sodium free	0	0	Choline chloride substituted for NaCl

0 no change; + or − small, non-significant change ($P = 0.1$); ++/−− a trend ($P = 0.05$ to 0·1); +++/−−− a significant change ($P = 0.05$ to 0·01); ++++/−−−− a significant change ($P < 0.01$).

Uptake at equilibrium was also measured for all inhibitors, the results of which are shown in Table 3. Similar to the initial rate results, the equilibrium uptake at reduced temperature (5°C) was statistically greater than the control ($P < 0.001$) for ibuprofen and indomethacin, again suggesting an adsorption phenomenon. Sodium azide did not show an effect on uptake, which agreed with the initial rate results (Table 3). Uptake in solutions containing 2,4-dinitro-phenol exhibited a small but statistically significant decrease for ibuprofen only compared with the control for equilibrium. The greatest difference between the initial rate and equilibrium results was observed with nigericin and sodium dependence.

Uptake at equilibrium in the presence of nigericin increased significantly ($P < 0.005$) over the control for both ibuprofen and indomethacin, whereas sodium dependence showed no effect at equilibrium. These results may not be an inhibitory effect but could be explained by the fact that the combination of nigericin with a high extracellular potassium concentration prevented the exchange of hydrogen ion for potassium ion across the cell, thus dissipating the proton gradient. The parietal cell, capable of excreting hydrochloric acid, could be affected by dissipation of the proton gradient, affecting the cell's ability to maintain its typical intracellular pH and therefore result in an instability of the biochemical events within the cell.

Determination of radiolabelled stability and metabolism of NSAIDs. The assay methodology used in this study was not specific for each NSAID, and so it was necessary to determine that uptake into parietal cells was occurring for the intact molecule. After incubation of either ibuprofen or indomethacin, chromatograms showed the existence of a single peak representing the intact molecule for both drugs. Significant metabolism was not considered likely since uptake over time through 15 min for the equilibrium plots showed a definite plateau and did not show any indication of a decrease.

Investigation of surface binding. Figure 3 shows an interaction of ibuprofen and indomethacin with the lysed cells which is similar in shape to what would

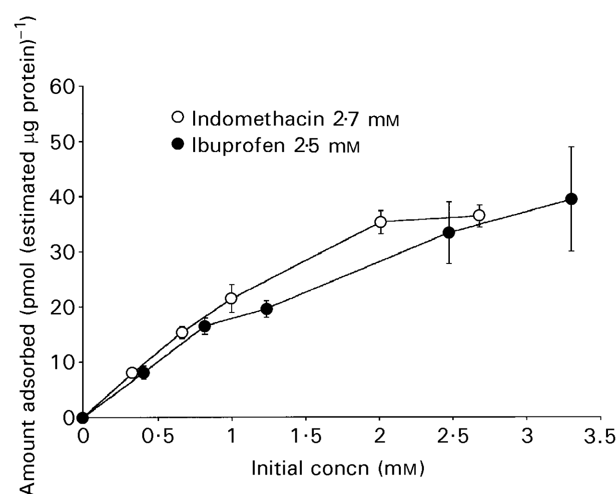

Figure 3. Indomethacin and ibuprofen interaction with lysed rabbit parietal cells. Each concentration is the mean ± s.d. of three measurements.

be expected for an adsorption interaction. In addition, both drugs showed nearly identical profiles with the lysed cells with respect to initial rate of uptake as well as equilibrium, suggesting that non-specific adsorption could be occurring. When ibuprofen and indomethacin were compared for uptake into intact parietal cells, indomethacin yielded an uptake (rate and saturation) 10-fold that of ibuprofen.

From the design of the experiment, it was not possible to differentiate whether adsorption occurred with the outer surface of the cells or with debris from the inside of the cell. It is yet to be determined if the adsorption is similar to binding observed with serum albumin or to what extent it may contribute to the overall uptake mechanism observed for intact cells.

Table 4 represents a summary of equilibrium uptake at 0·67 mM for ibuprofen, indomethacin and ketoprofen, and the lesion index in stressed rats published by Rainsford (1977). Although not enough data was generated to demonstrate a possible relationship with a measure of gastric toxicity, the plot suggests a trend such that it may be possible to predict cellular toxicity with NSAIDs by understanding uptake. The incompleteness of the Michaelis-Menten plots means that it was not possible to differentiate the contributions of active and passive transport. Likewise, with the increase in uptake at 5°C for ibuprofen and indomethacin, it is possible that adsorption was contributing to the uptake phenomenon.

Conclusions

Ibuprofen, ketoprofen and indomethacin all showed a rapid uptake with parietal cells isolated from the rabbit stomach mucosa. Initial rate as well as equilibrium data were consistent with Michaelis-Menten kinetics. However, because of the limited solubility of these agents, it was not possible to generate a complete profile. Overall, the results of the uptake experiments suggested that active transport, passive transport and adsorption contributed to the uptake mechanism.

Specifically, the initial shape of the Michaelis-Menten curves as well as the response of the NSAIDs to inhibitory conditions suggested that an active transport mechanism was involved in at least partially explaining the uptake of these agents. However, only nigericin showed a consistent effect; other inhibitory conditions were small and not statistically significant.

Uptake of ibuprofen and indomethacin increased with a lower medium pH, indicating that passive diffusion was also an important component to the drugs' uptake. This latter effect was evident from the overall ionization state of the drug as a function of pH. Adsorption was an additional mechanism that affected uptake for both ibuprofen and indomethacin. A decrease in temperature (5°C) increased uptake significantly, which is consistent with an observed rapid uptake.

The possible relationship between uptake at equilibrium and the lesion index in stressed rats means that physicochemical phenomena as well as biological phenomena may help to explain gastric toxicity associated with these agents.

Table 4. Relationship between uptake at equilibrium at 0·67 mM for indomethacin, ketoprofen and ibuprofen, and a gastric lesion index in rats.

Drug	Equilibrium uptake at 15 min (n = 3, mean ± s.d.)	Lesion index[a]
Ibuprofen	2·62 (0·481)	9
Ketoprofen	11·4 (0·435)	43
Indomethacin	59·1 (15·1)	49

[a]Data taken from Rainsford (1977).

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