J. Pharm. Pharmacol. 1996, 48: 119–135
Received April 21, 1995
Accepted April 28, 1995

Chemical Approaches to Improve the Oral Bioavailability of Peptidergic Molecules

JAMES SAMANEN, GLYNN WILSON*, PHILIP L. SMITH*, CHAO-PIN LEE*, WILLIAM BONDINELL, THOMAS KU, GERALD RHODES† AND ANDREW NICHOLS‡

*Departments of Medicinal Chemistry, *Drug Delivery, †Drug Metabolism, and ‡Pharmacology, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, PO Box 1539, King of Prussia, PA 19406, USA*

Abstract

This review discusses both tools and strategies that may be employed as approaches towards the pursuit of orally active compounds from peptidergic molecules. Besides providing a review of these subjects, this paper provides an example of how these were utilized in a research programme at SmithKline Beecham involving the development of orally active GPIIb/IIIa antagonists. The tools for studying oral drug absorption in-vitro include variants of the Ussing chamber which utilize either intestinal tissues or cultured epithelial cells that permit the measurement of intestinal permeability. Example absorption studies that are described are mannitol, cephalexin, the growth hormone-releasing peptide SK&F 110679 and two GPIIb/IIIa antagonist peptides SK&F 106760 and SK&F 107260. With the exception of cephalexin, these compounds cross the intestine by passive paracellular diffusion. Cephalexin, on the other hand, crosses the intestine via the oligopeptide transporter. Structure-transport studies are reviewed for this transporter. The tools for studying oral drug absorption in-vivo involve animals bearing in-dwelling intestinal or portal vein catheters. A study of the segmental absorption of SK&F 106760 is provided.

The review concludes with two chemical strategies that may be taken towards the enhancement of oral bioavailability of peptidergic molecules. The first strategy involves the chemical modification of peptides which enhance intestinal permeability, specifically the modification of amide bonds. The second strategy involves the design of compounds bearing nonpeptide templates, which are more amenable to the discovery of compounds with oral activity, from peptide pharmacophore models. An example is given regarding the discovery of SB 208651, a potent orally active GPIIb/IIIa antagonist, designed from the peptides SK&F 106760 and SK&F 107260.

Introduction: Peptide Drugs vs Nonpeptide Drugs

Differences in development of peptide drugs and nonpeptide drugs

Many drug discovery efforts today begin with a receptor that has been identified through the study of a pathological process using the tools of molecular biology. The endogenous ligand for these new therapeutic targets are quite often peptides or proteins (Samanen 1985), hence the ever increasing concern about peptidergic drugs and the attendant issue of oral bioavailability of peptidergic molecules.

The medicinal chemistry effort typically begins when a synthetic lead for the receptor has been identified through screening of biological samples containing natural products, proprietary databases, and more recently, combinatorial libraries (Jung & Beck-Sickinger 1992). In the absence of such discoveries one must begin with a peptide or protein fragment related to the endogenous ligand. A common method of affinity enhancement involves incorporation of peptide backbone conformational constraints (Toniolo 1989). These can be of a local nature in the form of modifications to individual amino acids (Toniolo 1989a), a regional nature in the form of γ- or β-turn mimetics (Toniolo 1991; Callahan et al 1993; Holzemann 1991), or a global nature in the form of functional groups which form cyclic structures (Samanen et al 1994). The resulting peptide drug may contain structural elements that are not related to the 20 native amino acids, but the relationship to the starting peptide is still discernable. Such compounds have been called semipeptides (Samanen et al 1994) or peptoids (Horwell et al 1990). Taken to the extreme, leads or drugs that bear no obvious relationship to the endogenous peptide ligand could be considered nonpeptides. Medicinal chemists are quite skilled at empirically developing semipeptide or nonpeptide drugs without relating them to the peptide through a pharmacophore model.

Whereas the nonpeptide drug typically arises from a template that has a good chance for oral activity, the peptide or semipeptide drug is derived from a peptidic template, which is not orally active, and the incorporation of sufficient modifications to engender oral activity remains a challenge (Kleeman et al 1992; Rosenberg et al 1993a, b; Hashimoto et al 1994). Fig. 1 shows how the processes for peptide and nonpeptide drug development may be interlinked through the determination of a pharmacophore model. An example of this approach will be given in this review from work at SmithKline Beecham on GPIIb/IIIa antagonists. This work explores an approach to drug design in which one may design nonpeptide templates (Ku et al 1993) that have a greater potential for oral activity from a peptide pharmacophore model that is developed through the evaluation of the conformations of highly constrained peptides. At SmithKline Beecham, work that began with a low-affinity

Correspondence: J. Samanen, Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, PO Box 1539, King of Prussia, PA 19406, USA.

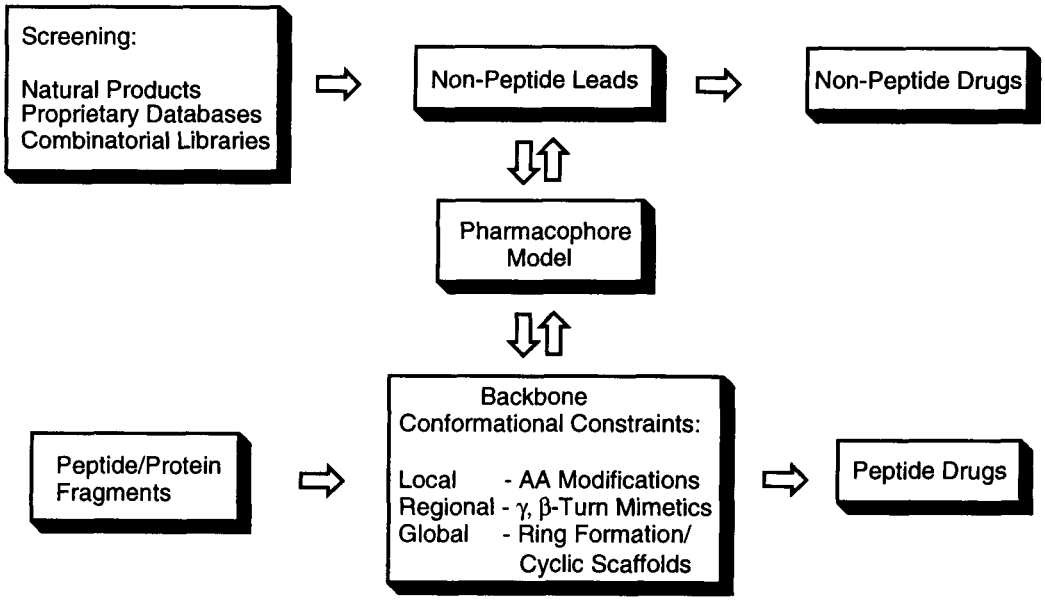


FIG. 1. Drug discovery paradigm.

peptide fragment to fibrinogen led to the discovery of the high-affinity peptide SK&F 107260 (Ali et al 1992, 1994; Samanen et al 1991) which served as the template for the design of the potent orally active nonpeptide antagonist SB 208651 (Bondinell et al 1994).

The pursuit of oral GPIIb/IIIa antagonists and thrombotic agents for the prevention of myocardial infarction and stroke. It is important to initially review how the two potent intravenous GPIIb/IIIa antagonists SK&F 106760 and SK&F 107260 were discovered. The role of GPIIb/IIIa antagonists in the prevention of thrombosis has been amply reviewed (Nichols et al 1992, 1994a). Since chronic oral administration of GPIIb/IIIa antagonists could, in principal, address leading causes of death in the United States (Gunby 1992) intense interest has developed over the discovery of orally active GPIIb/IIIa antagonists. Several groups discovered three fragments that inhibit fibrinogen—GPIIb/IIIa interaction (Fig. 2); the dodecapeptide from the C-terminus of the γ chain (Kloczewiak et al 1984, 1989); and

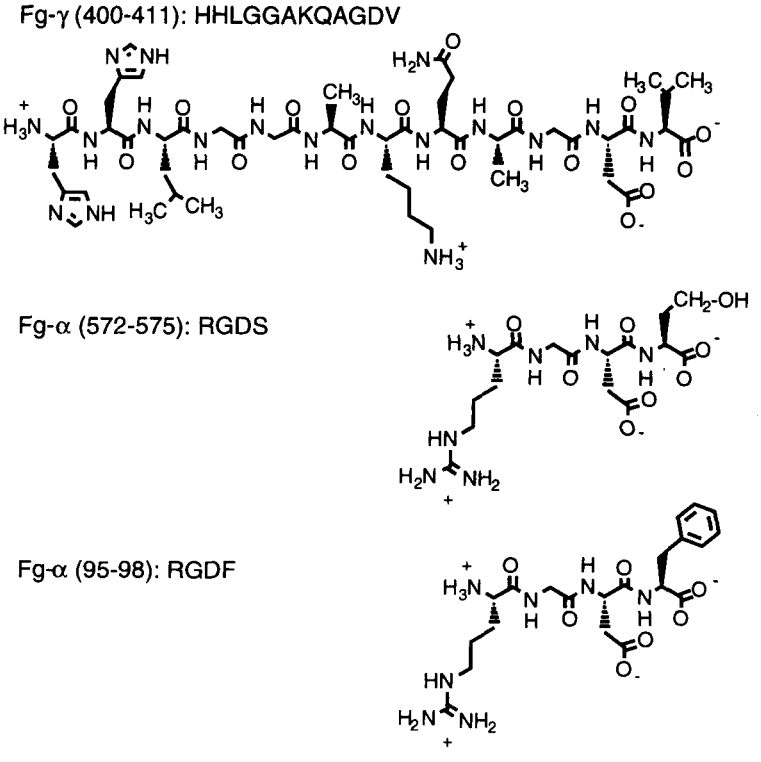


FIG. 2. Inhibitory peptides derived from fibrinogen.

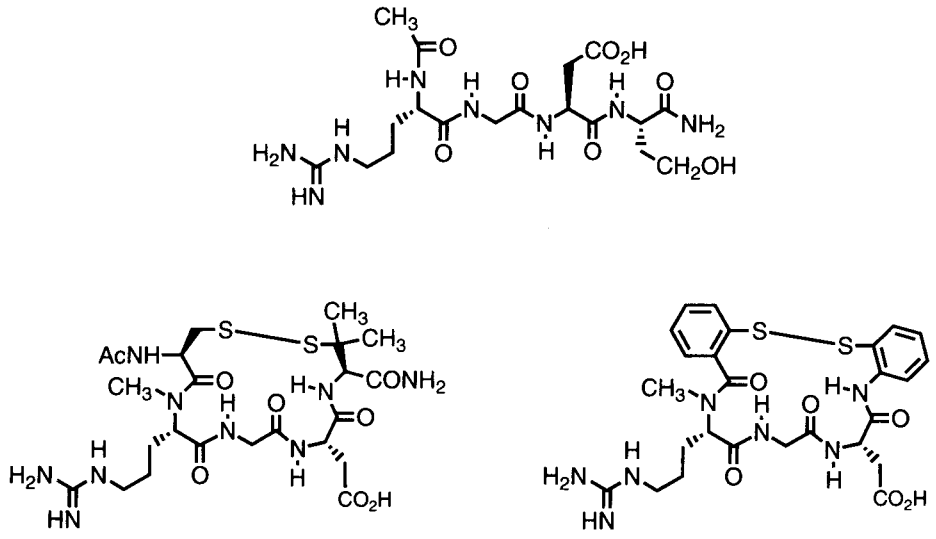


FIG. 3. Structures of Acetyl-RGDS-NH_2, SK&F 106760 and SK&F 107260.

two tetrapeptides from the alpha chain (Gartner & Bennett 1985; Ginsberg et al 1985; Plow et al 1987; Andrieux et al 1989), both of which contain the tripeptide sequence Arginyl-Glycyl-Aspartic acid, or RGD, which is the minimum binding epitope in a number of integrin-mediated cell adhesion processes (Smith 1994). In contrast to fibrinogen, which binds to GPIIb/IIIa with a K_d of 43 nM, all of these peptides bind to GPIIb/IIIa with low affinity, in the micromolar range. The tetrapeptide was selected as a starting point for synthetic investigations, since it was smaller and displayed greater affinity in-vitro than the γ-chain peptide. Beginning with acetyl-Arg-Gly-Asp-Ser-amide, a search was conducted for high affinity peptide analogs, which culminated in the discovery of two cyclic peptides, SK&F 106760 and SK&F 107260 (Fig. 3) (Samanen et al 1991; Ali et al 1992, 1994). In SK&F 106760 the cyclic structure came about through incorporation of a disulfide tether consisting of an N-terminal cysteine and a C-terminal penicillamine. In SK&F 107260 the disulfide tether consisted of a diaryldisulfide. Table 1 compares in-vitro data for the two peptides with acetyl-Arg-Gly-Asp-Ser-amide. Employing ^{125}I-fibrinogen as the radioligand in a binding assay with human GPIIb/IIIa, SK&F 106760 was found to display high affinity (K_i 0.058 μM), matching the affinity of endogenous fibrinogen (K_i 0.043 μM). With SK&F 107260 even further enhancement in affinity was achieved (K_i 0.002 μM). Tritiated SK&F 107260 was subsequently employed as the radioligand in the binding assay, because it avoided problems associated with reproducible preparation of radioiodinated fibrinogen. It is readily apparent that the rank orderings of affinity are identical in both assays. Platelet aggregation was performed with either canine or human platelet rich plasma, stimulated to aggregate with 10 μM ADP. Both peptides SK&F 106760 and SK&F 107260 were potent inhibitors of platelet aggregation (IC50 0.360 μM and 0.090 μM, respectively). The IC50 values follow the rank order of affinities suggesting that the anti-aggregatory activities of these compounds may be due to their abilities to inhibit fibrinogen—GPIIb/IIIa binding.

The in-vivo efficacy of GPIIb/IIIa antagonists can be readily appraised in conscious dogs by studying platelet aggregation ex-vivo. When SK&F 106760 was administered by a short intravenous infusion at 1 mg kg^{-1} a rapid inhibition of ex-vivo platelet aggregation occurred (Fig. 4), which lasted for a period of about 40 min. At higher doses, increases in the duration of inhibition were observed. When SK&F 106760 was administered at 3 mg kg^{-1} by bolus intraduodenal administration, a slow onset of inhibition of ex-vivo platelet aggregation was observed, which reached a maximal inhibition of ca 40% at approximately 60-195 min after administration (Nichols et al 1994b). The same results were observed for SK&F 107260 (unpublished). Other studies with these peptides have shown that they were potent and fully efficacious antithrombotic agents in several

Table 1. In-vitro activity of acetyl-RGDS-NH_2, SK&F 106760 and SK&F 107260.

Compounds	Anti-aggregation Canine PRP/ADP[a] IC50 (μM)	Binding inhibition Human GPIIb/IIIa[b] ^{125}I-Fg K_i(μM)	[^{3}H]SK&F 107260 K_i(μM)
Acetyl-RGDS-NH_2	91·30	4·20	37·0
SF&F 106760	0·360	0·058	0·15
SF&F 107260	0·090	0·002	0·004

[a] Inhibition of platelet aggregation induced by ADP in canine platelet-rich plasma. [b] Inhibition of ^{125}I-fibrinogen or [^{3}H]SK&F 107260 binding to GPIIb/IIIa purified from human platelets, reconstituted in liposomes.

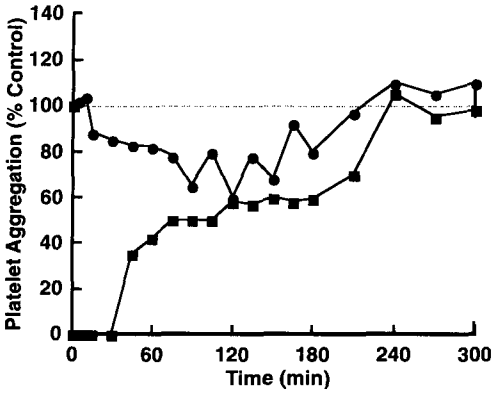


FIG. 4. Inhibition of ex-vivo platelet aggregation by SK&F 106760 administered at 1 mg kg^{-1} by intravenous administration (■) and 3 mg kg^{-1} by intraduodenal administration (●) (Nichols et al 1994).

animal models of thrombosis (Nichols et al 1994). At issue, was the lack of adequate oral activity.

Barriers to Oral Drug Delivery

Any orally administered drug encounters a number of biological barriers before it reaches the target cell (Fig. 5). At each barrier, some drug is lost due to incomplete absorption or metabolism. Oral drugs face the epithelial barrier of the gastrointestinal tract and the liver, which receives the venous circulation from the gastrointestinal tract. Following passage through the liver, oral and intravenous drugs encounter the same obstacles: equilibration with tissue, irreversible uptake in the kidney, and degradation in the lung and the capillary endothelium. CNS drugs must also pass through the blood brain barrier, which has been addressed by Begley in this Symposium.

Tools for Studying Oral Drug Absorption

The challenge of developing oral peptides has encouraged scientists to examine the process of intestinal absorption and first-pass clearance in great detail. In so doing, they have developed what have become tools for studying these phenomena in the form of in-vitro asays which utilize intestinal or liver preparations and culturable cell lines, such as Caco-2 cells and in-vivo assays (Smith 1995). These tools can be employed as screens alongside other in-vitro and in-vivo assays to help the medicinal chemist select candidate compounds as potential oral agents. Examples will be given from the laboratories at SmithKline Beecham and from other laboratories (see below) where these tools were developed.

Fig. 6 considers the various potential pathways for peptide absorption across the intestinal epithelium (Smith et al 1992). The most common route, on the left, is the paracellular route (Route 1). It is a passive diffusional path that is taken by small hydrophilic molecules, that pass through the tight junctions between adjacent epithelial cells. Routes 2a–2d are transcellular pathways. An important pathway (2a) involves the carrier-mediated active transport of di- and tripeptides (Smith et al 1993). A second diffusional pathway (2b) is taken by small lipophilic molecules. Pathway 2c involves the receptor-mediated transcytosis of proteins, which bind to a receptor complex on the surface. The complex forms vesicles which move to the basolateral surface and ultimately release the protein at the basolateral surface. This process is a low-capacity process and is therefore not likely to be routinely exploitable. Pathway 2d involves cellular uptake and processing via the endoplasmic reticulum and Golgi apparatus, a process employed for receptor recycling and lipid absorption. Its low capacity makes it unlikely to be routinely exploitable. This review

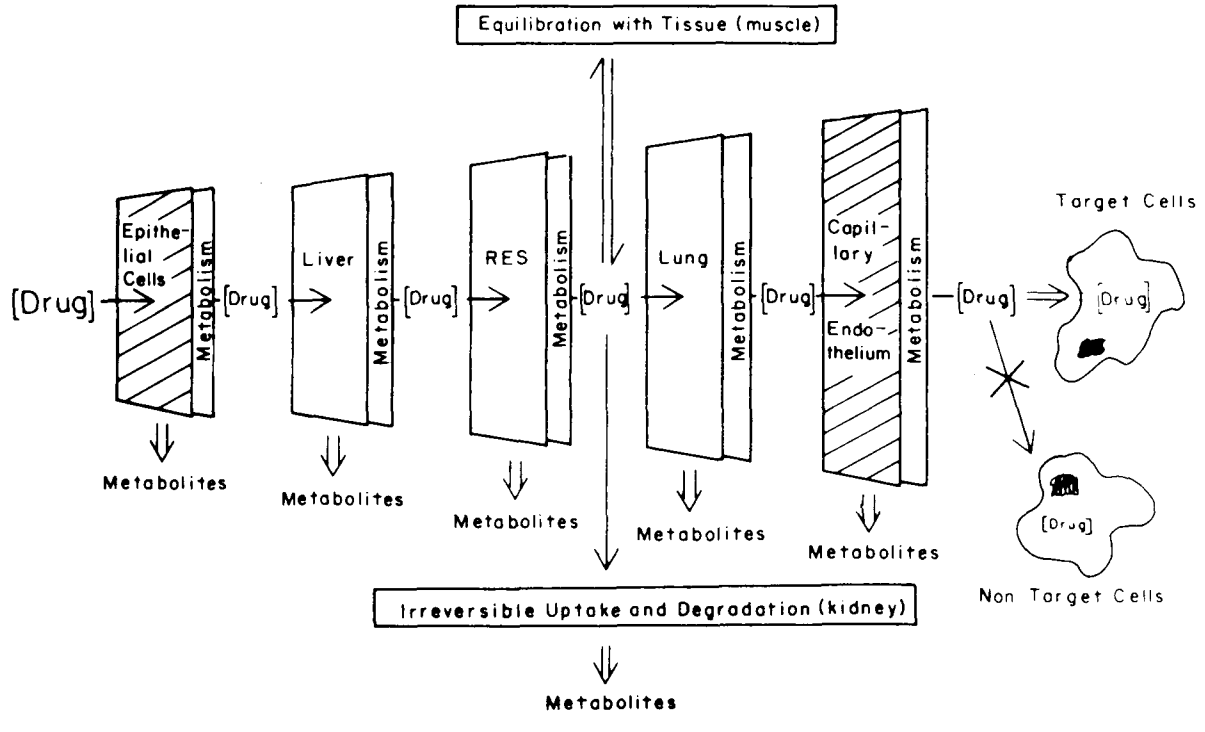


FIG. 5. Biological barriers to drug absorption.

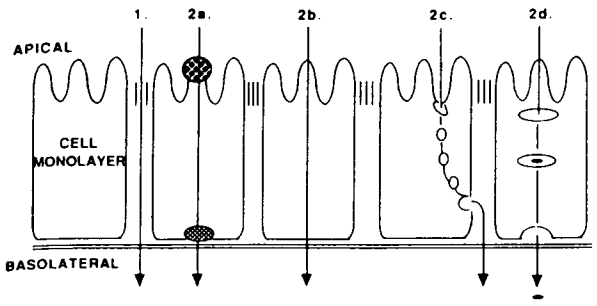


FIG. 6. Barriers to intestinal absorption of an oral drug (Smith et al 1992). 1. Paracellular route (between tight junctions). 2. Transcellular routes: 2a. carrier-mediated active transport; 2b. passive diffusion across cell membranes; 2c. receptor-mediated transcytosis within vesicles; 2d. cellular uptake and processing via endoplasmic reticulum and Golgi apparatus.

will focus on the two diffusional pathways 1 and 2b and the carrier-mediated pathway 2a.

A second major concern regarding intestinal absorption of peptides, has to do with metabolism within the lumen, the intestinal brush border and inside the epithelial cells (Matuszewska et al 1988; Smith et al 1992). A variety of peptidases has evolved to remove N-terminal amino acids, C-terminal amino acids and dipeptides, as well as other peptidases which select certain amino acids in a sequence for peptide chain cleavage at that point. A variety of in-vitro systems are available for the study of peptide metabolism. Many of the enzymes are commercially available, permitting one to study the metabolic stability of a peptide. If one wishes to enhance the intestinal absorption of a peptide, one should first select a peptide analogue that is known to be stable to enzymatic hydrolysis to eliminate that as a potential barrier to absorption.

In-vitro assays

Utilization of in-vitro assays for evaluation of intestinal permeability.

The Ussing chamber for measuring intestinal permeability. A commonly employed apparatus for studying intestinal absorption is the Ussing chamber (Ussing & Zerahn 1951), variants of which can be employed to study either intestinal tissues or cultured cells, such as Caco-2 cells (see references in Smith et al 1992). Duodenal, ileal, jejunal or colonic mucosa are stripped of underlying muscle and mounted in a chamber, such as the one shown in Fig. 7 (Smith et al 1992). This creates a bicompartmental system in which both the mucosal and serosal surfaces are bathed with oxygenated buffer solution. Epithelial integrity is determined from both the transepithelial electrical resistance across the tissue and the permeability of a marker molecule such as mannitol (Marks et al 1991; Swaan et al 1994). One can add a solute to either compartment and follow its rate of appearance in the other compartment by standard means of chemical detection.

Permeability of mannitol, an example of paracellular passive diffusion. By sampling over a period of time one can determine the rate of transport or flux across the epithelium. Fig. 8 displays the rate of transport of mannitol (Hidalgo et al 1993; Swaan et al 1994), an orally available hydrophilic molecule, across rabbit ileum, jejunum and distal colon. When rabbit ileum is employed in the chamber and mannitol is placed in the mucosal compartment, its appearance in the serosal compartment with time is linear, after a 15-min period and this m-to-s flux is 0.18%/3 cm^2. When mannitol is placed in the serosal compartment and its appearance is followed in the mucosal compartment, the s-to-m flux is identical, or 0.17%/3 cm^2. This equality of s-to-m and m-to-s fluxes is also observed in jejunum and distal colon. These are the hallmarks of a hydrophilic solute that traverses the intestine by paracellular passive diffusion.

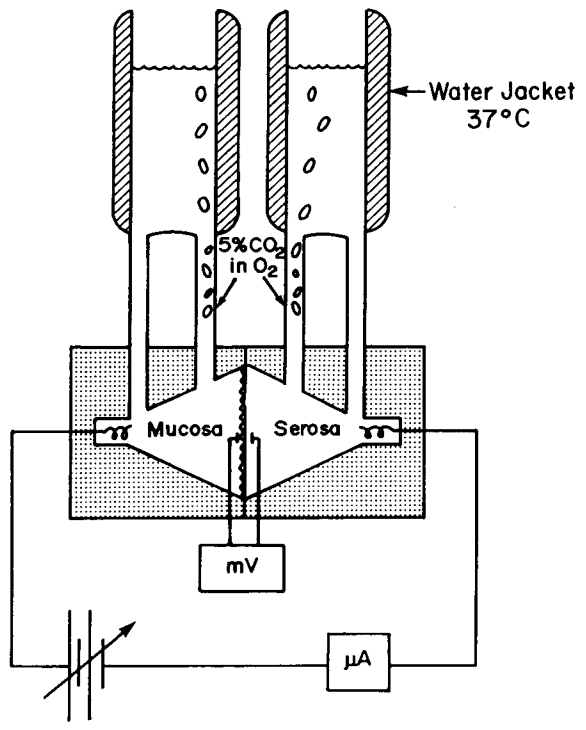


FIG. 7. The Ussing chamber for the study of transepithelial transport.

Permeability of cephalexin, an example of carrier-mediated transport. In Fig. 9, the experiment is repeated with the

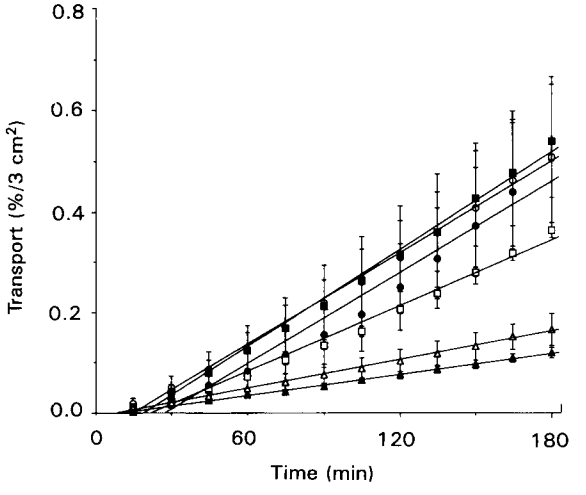


FIG. 8. Intestinal permeability (cm h^{-1}) of mannitol (Hidalgo et al 1993). Rabbit ileum (●) m → s, 0·008, (○) s → m, 0·007, rabbit jejunum (■) m → s, 0·010, (□) s → m, 0·008, rabbit distal colon (▲) m → s, 0·002, (△) s → m, 0·002.

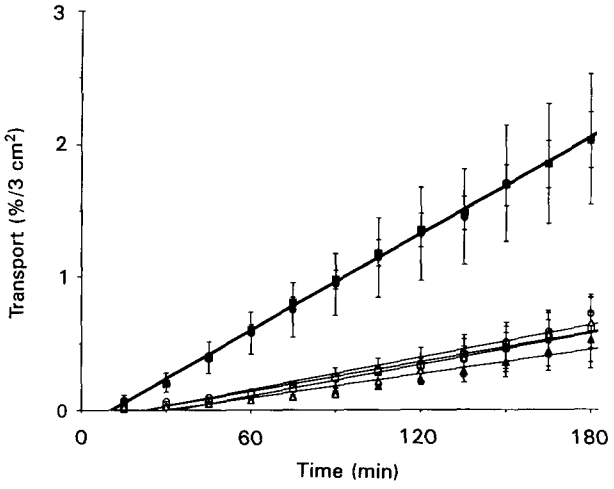


FIG. 9. Intestinal permeability (cm h^{-1}) of cephalexin (Hidalgo et al 1993). Rabbit ileum (●) m → s, upper set 0.028, (○) s → m, lower set 0.010; rabbit jejunum (■) m → s, upper set 0.028, (□) s → m, lower set 0.009; rabbit distal colon (▲) m → s, lower set 0.008, (△) s → m, lower set 0.009.

β-lactam antibiotic cephalexin (Hidalgo et al 1993). In this experiment, we find that the m-to-s flux is three times greater than the s-to-m flux for both the rabbit ileum and the rabbit jejunum. With the distal colon, m-to-s flux and s-to-m fluxes are identical. Thus, in the small intestine, something other than passive diffusion must be occurring. In Fig. 10, the m-to-s flux for cephalexin, shown in closed bars, is compared with the m-to-s flux for mannitol, shown in open bars and these fluxes are evaluated at different pH levels (Hidalgo et al 1993). Panel A shows that the m-to-s flux in the rabbit ileum is enhanced at acid pH for cephalexin but not for mannitol. The m-to-s flux for either compound in the distal colon is the same at either pH 5.5 or 7.4. Panel B, shows that the s-to-m flux in rabbit ileum is identical at all pH levels for both compounds. These results are consistent with transport of cephalexin by the carrier-mediated pH-dependent transport system present in small but not large intestinal mucosa (Ganapathy & Leibach 1985); the presence of a greater m-to-s than s-to-m flux in the small intestine is characteristic of molecules transported by carrier-mediated processes. Molecules such as cephalexin, arginine and 5-fluorouracil also demonstrate energy dependence and are therefore described as actively transported (Smith et al 1988; Swaan et al 1994).

Permeability of SK&F 110679, SK&F 106760 and SK&F 107260, examples of peptide transport by paracellular passive diffusion. An example of the intestinal absorption of a peptide is SK&F 110679, a hexapeptide which releases growth hormone in-vitro (Bowers et al 1980) and after intravenous, or respiratory administration (Smith et al 1994). Although SK&F 110679 is orally active, the comparison of equi-effective intravenous and oral doses in rats suggests a bioavailability of 0.7% (Walker et al 1990). In either rabbit duodenum, on the left or the rabbit jejunum, on the right, the m-to-s and s-to-m fluxes for SK&F 110679 are identical, and less than the corresponding mannitol fluxes, Fig. 11 (Smith et al 1994) which suggests that absorption occurs by passive diffusion. This experiment suggests that the lack of adequate oral bioavailability for SK&F 110679 is due to its low intestinal absorption.

For the GPIIb/IIIa antagonists SK&F 106760 and SK&F 107260, a pharmacokinetic analysis of the intravenous and intraduodenal experiments in the dog revealed low bioavailabilities of 3.9% for SK&F 106760 and 3.3% for SK&F 107260 (Table 2). The intravenous activities observed for both compounds suggests that these peptides are relatively stable to enzymatic hydrolysis. The intestinal permeabilities were lower than mannitol, however (Table 2). The m-to-s and s-to-m fluxes were not pH-dependent and were identical. Thus, it appears that these compounds cross the intestinal wall by passive diffusion, and have low permeability. Support for this conclusion is provided by the finding that intestinal absorption of SK&F 106760 is enhanced by

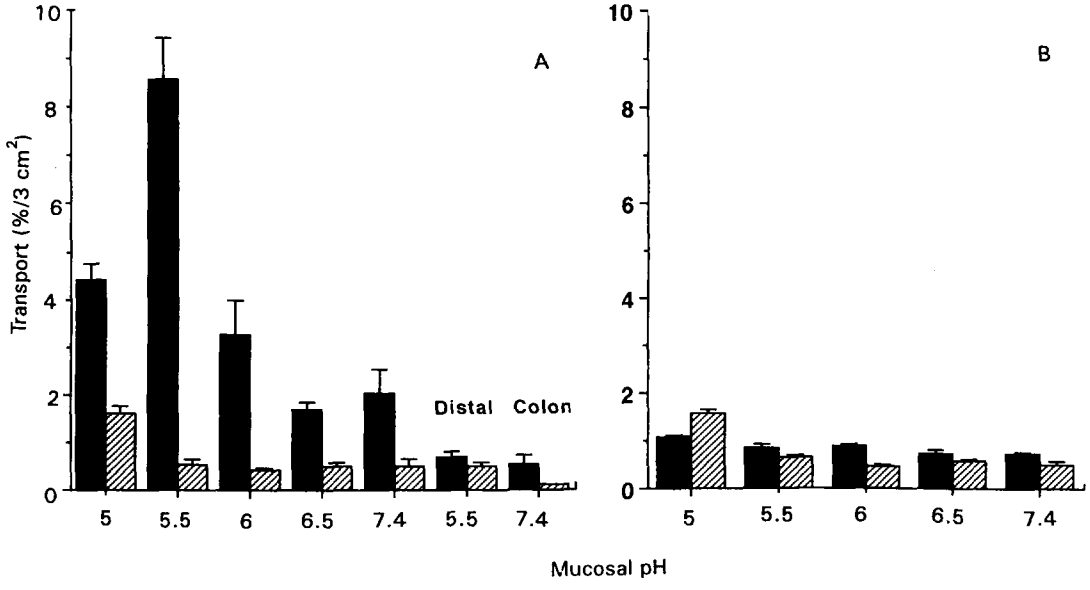


FIG. 10. Intestinal permeability: comparison of pH-dependent permeability for cephalexin but not mannitol (Hidalgo et al 1993). A. m → s cephalexin ■, pH dependent; mannitol ▨; B. s → m cephalexin ■, mannitol ▨.

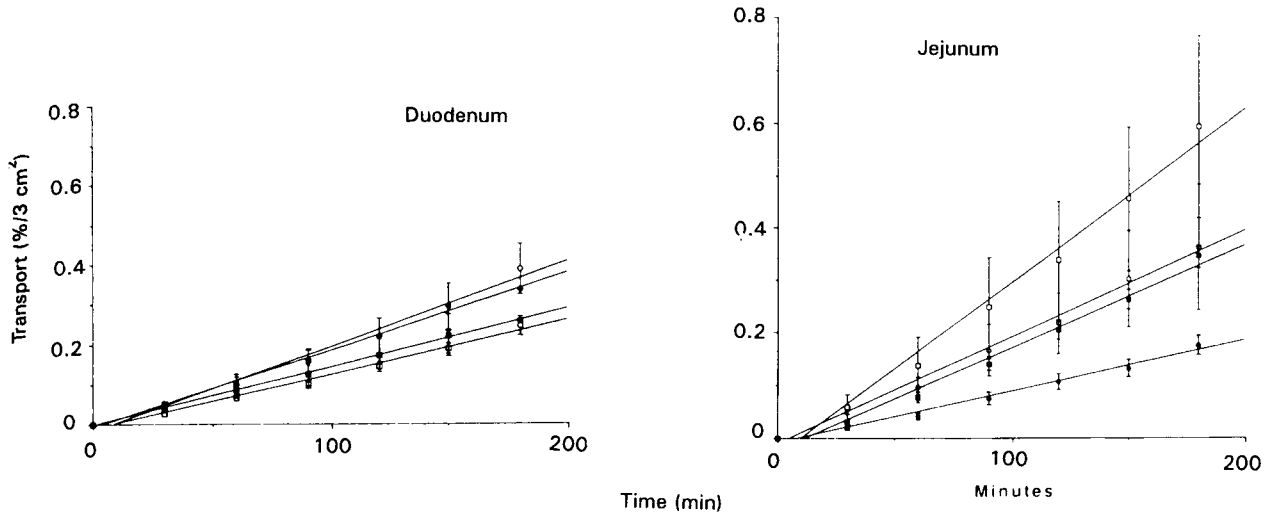


FIG. 11. Intestinal permeability (cm h^{-1}): Comparison of SK&F 110679 with mannitol (Smith et al 1994). A. Rabbit duodenum, SK&F 110679 (○), m → s 0.002, (●) s → m 0.004, mannitol (□) m → s 0.005, (■) s → m 0.006. B. Rabbit jejunum, SK&F 110679 (○) m → s 0.004, (●) s → m 0.002, mannitol (□) m → s 0.008, (■) s → m 0.004.

medium-chain glycerides which have been demonstrated to increase intestinal permeability for a number of molecules (Constantinides et al 1994; Yeh et al 1994).

Studies of the oligopeptide transporter. Cephelaxin as a tool for evaluation of the oligopeptide transporter. The di/tripeptide transporter merits further discussion. Fig. 12 summarizes the biochemistry of the system (Smith et al 1993). The pH gradient that favours absorption by the transporter, is generated by the combined action of the brush border membrane sodium/potassium exchanger, and a basolateral sodium/potassium ATPase. Transport of peptide substrate (S) is accompanied by proton transport. Inside the cell, peptide (S) is hydrolysed to its constituent amino acids which are then pumped out of the cell via appropriate basolateral amino acid carriers. There may also be a di/tripeptide transporter in the basolateral membrane to pump out of the cell peptide substrate that was not hydrolysed (Thwaites et al 1993). Of course, a topic of concern to medicinal chemists is the substrate specificity of this di/tripeptide transporter. Fortunately, its specificity is relatively low, since many β-lactam penicillin and cephalosporin antibiotics, ACE inhibitors, TRH analogues and renin inhibitors are transported by this carrier (Smith et al 1993). There is a wide variety of systems that have been employed in the study of the di/tripeptide transporter. These have been reviewed elsewhere (Smith et al 1993). At SmithKline Beecham it has been advantageous to employ epithelial cell lines that maintain viability in cell culture, can be grown to a confluent monolayer, display polar character and contain carrier systems that have been described in the human small intestinal epithelium. Caco-2 cells, in particular, have been utilized extensively at SmithKline Beecham (Dantzig & Bergin 1988, 1990).

In-depth analysis of cephalexin transport in this system revealed two components to total transport (Fig. 14) (Gochoco et al 1994). Total transport is shown in the open boxes. Linear passive diffusion is shown in closed triangles, and saturable, active transport is shown in closed boxes. Saturation would not be observed generally in-vivo due to the very large surface area of the intestinal tract. To study oligopeptide transporter specificity, cephalexin may be employed as the standard, since it is an orally absorbed cephalosporin which has been shown to be absorbed by the di/tripeptide transporter. With tritiated cephalexin one can then look at compounds that may compete with cephalexin for uptake by the transporter.

Table 2. Rabbit ileum permeability and intraduodenal bioavailability of SK&F 106760, SK&F 107260 and mannitol.

	Rabbit ileum permeability (cm h^{-1})	Intraduodenal bioavailability (% ±s.d.)
SK&F 106760	0·0015	3·9 ± 2·2 (dog, Nichols et al 1994)
SK&F 107260	0·0030	3·3 ± 2·8 (dog)
Mannitol	0·019	20 (man, Laker et al 1992)

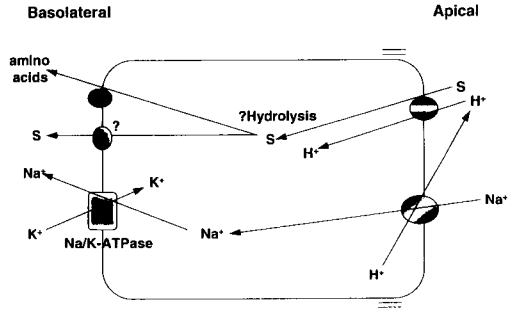


FIG. 12. Scheme depicting the dipeptide transporter. Scheme depicts the transport of peptide substrate (S) across the apical and basolateral cell membranes via peptide transporter proteins, with accompanying proton transport, involvement of a brush border membrane sodium/potassium exchanger, and a basolateral sodium/potassium ATPase, along with hydrolysis of S to constituent amino acids.

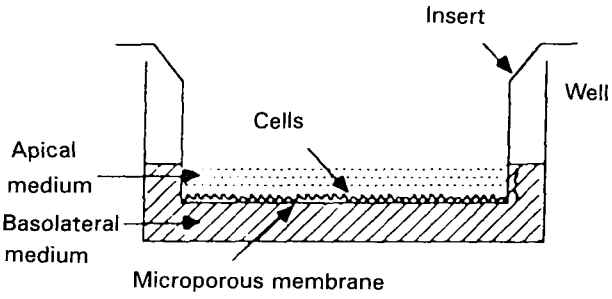


FIG. 13. Technique employed for studying uptake across the apical cell membrane (Audus et al 1990).

Substrate specificity: range of compounds, SAR. Other β-lactam antibiotics compete with cephalexin for binding to the transporter: cefadroxil, ampicillin, penicillin G, and dipeptides such as Gly-Pro and Pro-Gly, but not the constituent amino acids glycine or proline or other amino acids shown in the insert (Fig. 15) (Gochoco et al 1994)). The carrier is somewhat sensitive to chirality. Although Val-D-Val and D-Val-Val show the same level of inhibition as Val-Val, D-Val-D-Val does not (Table 3) (Hidalgo et al 1995). Substrate specificity has also been extended to cyclic vs acyclic peptides (Fig. 16) (Hidalgo et al 1995). While linear Gly-Pro and Ala-Ala bind to the transporter, cyclo-(Gly-Pro) and cyclo-(Ala-Ala) do not. Cyclo-(Asp-Gly), cyclo-(Asp-Phe), cyclo-(Asp-Asp), and cyclo-(Glu-Glu), however, bind to the transporter. Further investigation revealed that the common element in all these ligands for the transporter was the presence of least one carboxylate group, and that it does not matter whether the carboxylate group resides at the C-terminus or on a side chain.

Although these results demonstrate interaction by a variety of substrates with the transporter, additional transport experiments should be conducted since there are ligands which bind to the transporter but do not appear to be transported across the eipthelial barrier (Hidalgo et al 1995).

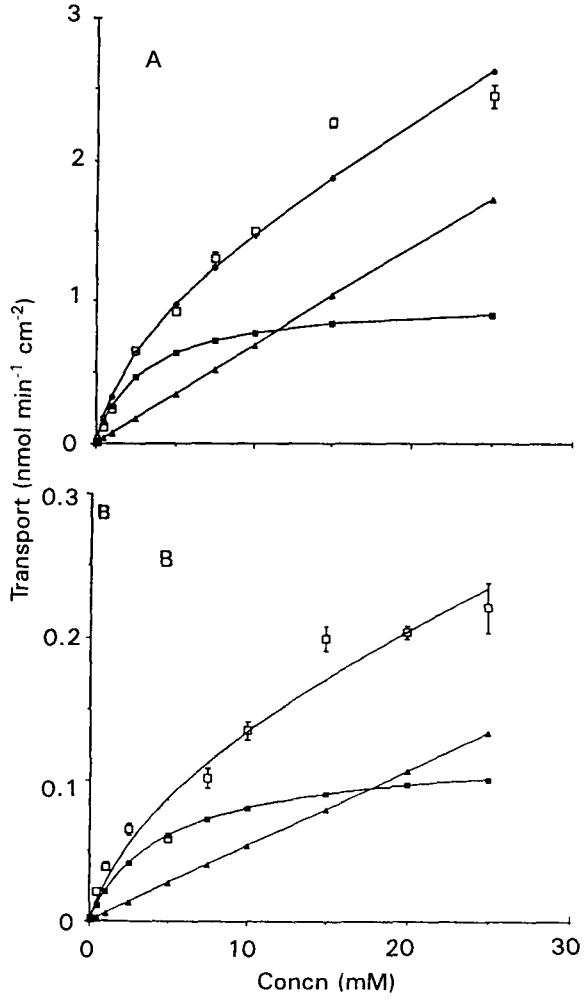


FIG. 14. Concentration-dependent transport of cephalexin across Caco-2 cells (Gochoco et al 1994). Transport of cephalexin (apical pH 6.0) across Caco-2 monolayers mounted in diffusion chambers determined over a 90-min time course. Drug is expressed as nmol $min^{-1}cm^{-2}$ (± s.e.). Open boxes = total transport, closed triangles = passive diffusion, closed boxes = carrier-mediated transport.

Transcellular passive diffusion and apical efflux. Caco-2 cells can also be employed in the study of transcellular passive diffusion across the epithelium. Many workers have found compounds where basolateral to apical flux is greater than the expected apical to basolateral flux. This apparent secretory mechanism has been attributed to an apical efflux process. This type of a process appears to be driven by an apical transporter called P-glycoprotein. Hunter et al (1991) have shown in the MDCK cell line that tritiated vinblastine suffered greater efflux than absorption. They showed that the apical P-glycoprotein actively pumps vinblastine back into the lumen at a greater rate than the normal rate of passive diffusion, resulting in a net apical efflux (Fig. 17). This apical efflux mechanism may explain why simple enhancements in lipophilicity may not always result in enhanced absorption.

Utilization of in-vitro assays for evaluation of liver clearance. A second hurdle for oral drugs after the gastrointestinal tract, of course, is the liver. There are a number of in-vitro assay systems that have been employed for evaluating liver clearance. These systems will not be reviewed in this paper, but suffice it to say that the in-vitro systems may be somewhat artifactual, in that more metabolic transformations may be observed in-vitro than would be found in-vivo. On the other hand, human in-vitro systems could be more predictive of human metabolism than an in-vivo animal model.

In-vivo assays

Intestinal cannulation. This section considers in-vivo assay systems for studying intestinal absorption and first-pass clearance. The critical component in these animal models is the in-dwelling catheter (Meunier et al 1993). It is

Table 3. Effect of dipeptide chirality on cephalexin uptake by Caco-2 cell monolayers (Hidalgo et al 1995).

Inhibitor (20 mM)	Inhibition (%)
Val-Val	82 ± 1·1
Val-D-Val	77 ± 0·8
D-Val-Val	80 ± 1·1
D-Val-D-Val	12 ± 1·7

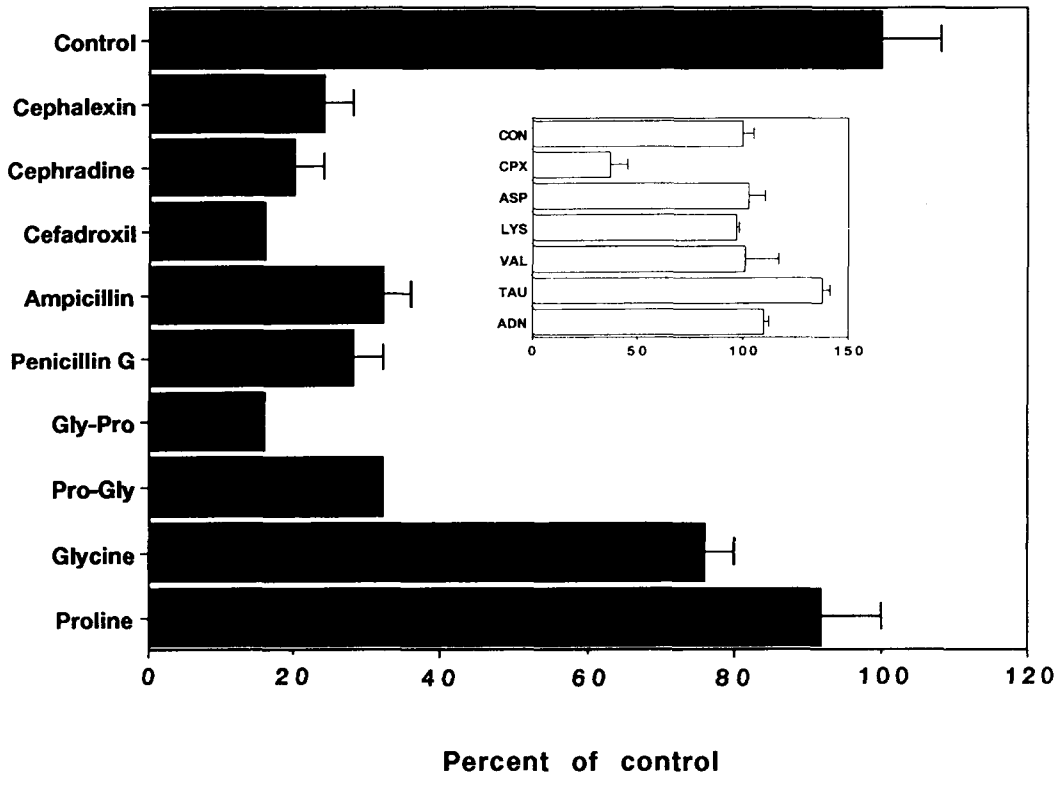


FIG. 15. Inhibitors of cephalexin transport with amino acids, β-lactams, dipeptides (Gochoco et al 1994). Inset: inhibition with amino acids and other carrier substrates.

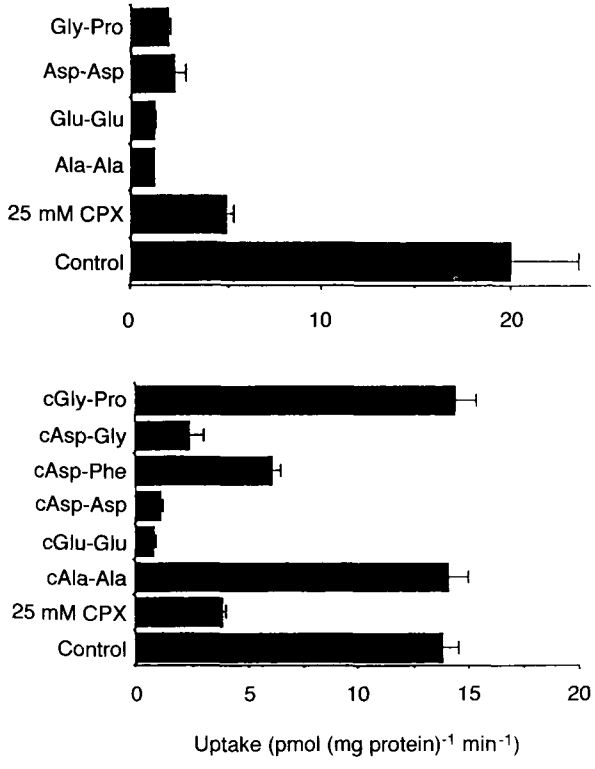


FIG. 16. Effect of cyclization of dipeptides on uptake of cephalexin by Caco-2 cell monolayers (Hidalgo et al 1995).

implanted into the upper right flank of a dog. The attached catheter is inserted into the intestinal tract and sutured tightly to prevent leakage. Dye-contrast imaging is employed to ensure proper placement of the catheter and to ensure that there is no leakage. In bypassing the stomach, one eliminates the variables of gastric emptying, stomach pH and vomiting. The catheter can be placed in different segments of the gastrointestinal tract to study variations in absorption.

SK&F 106760 by intravenous, intraduodenal, intrajejunal, and intracolonic administration. The GPIIb/IIIa antagonist SK&F 106760 serves as an example of the evaluation of segmental absorption in-vivo (Nichols et al 1994). Its

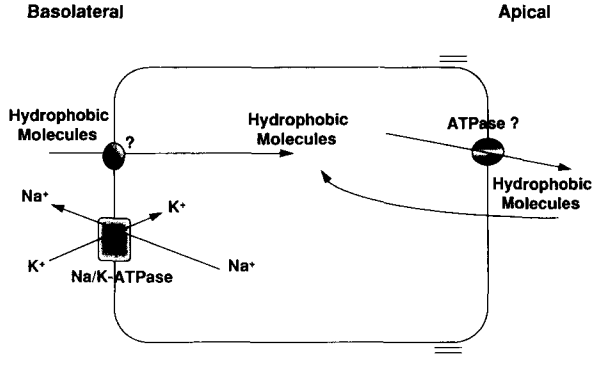


FIG. 17. Caco-2 cells for study of oligopeptide transporters. Apical efflux.

presence in plasma is detected pharmacologically by measuring ex-vivo platelet aggregation. Intravenous administration of 1.0 mg kg^{-1} SK&F 106760 gave rise to a rapid inhibition of ex-vivo platelet aggregation, which lasted for about 40 min (Fig. 18). Intraduodenal and intrajejunal administration of SK&F 106760 at 3 mg kg^{-1} gave weak responses (Fig. 18). Pharmacokinetic analysis of the plasma levels following intraduodenal or intrajejunal administration revealed bioavailabilities of about 3 and 6%, respectively. If SK&F 106760 is adminstered intracolonically, however, no pharmacological response was observed (data not shown). The residence time of a compound in the colon is considerably longer than in other segments of the intestinal tract. There is also little hydrolysis of peptides in the colon. Thus, had a good response been observed from intracolonic administration, a modified release formulation (Saffran et al 1986) might have been considered to increase bioavailability of SK&F 106760. Since there appeared to be no absorption of the peptide in the colon, deployment of this strategy would not be called for.

Portal-vein cannulation. The in-dwelling catheter can also be placed into the portal vein (Swaan et al 1994) that receives the venous circulation from the intestinal tract and conducts the venous blood into the liver, so-called first-pass clearance. Thus, if drug is given intraportally one can bypass the intestinal tract to examine the effects of first pass clearance in the absence of intestinal absorption. This technique was used to good effect in the study of SK&F 110679 (Smith et al 1994). If one cannulates the bile duct, one can also measure metabolites and obtain a complete analysis of liver clearance. Thus intraportal studies can be as valuable as intraintestinal studies.

Chemical Modifications which Enhance Intestinal Permeability

Amide-bond modifications

Besides the several tools available for the study of oral absorption, it is important to consider the types of chemical strategies that are available to the medicinal chemist in the design of peptides with enhanced oral bioavailability. With the tools just described, medicinal chemists could potentially screen compounds and develop a structure-permeability relationship to more readily design analogues with enhanced intestinal permeability. The group at Upjohn (Conradi et al 1991, 1992; Karls et al 1991; Burton et al 1992, 1993) have studied series of peptides, and have begun to make some important observations concerning the factors which influence intestinal permeability. Fig. 19 shows a series of peptides **I-V**, analogues of the tripeptide **I**, Ac-Phe-Phe-Phe-NH_2, that display different rates of permeability across Caco-2 cells (Conradi et al 1991). Investigation of the factors which might correlate with these increases led to the finding that octanol/water partition coefficients do not correlate with intestinal permeability. They discovered, however, in the series of analogues **II-V**, which contain an increasing number of *N*-methylated amide bonds, an inverse correlation between intestinal permeability and the number of potential hydrogen bonds that could occur with the peptides. Peptide **V**, containing all *N*-methylated amide bonds, displayed the highest intestinal permeability, whereas the others displayed decreasingly lower intestinal permeabilities. They hypothesize that intestinal permeability is increased by decreasing the desolvation energy that is required to remove a peptide from the aqueous environment to the lipid environment of the epithelial cell membrane.

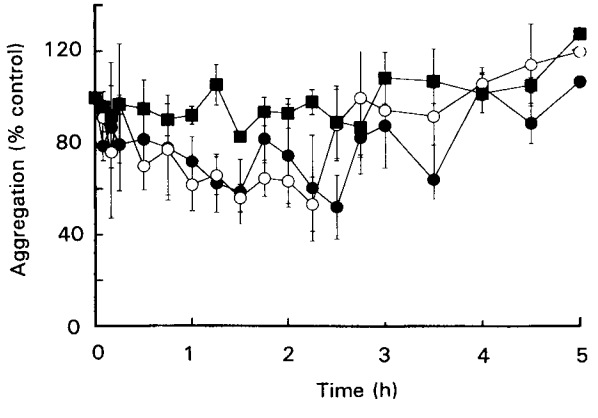


FIG. 18. Inhibition of ex-vivo platelet aggregation (Nichols et al 1994) by SK&F 106760 after intraduodenal (●), intrajejunal (○), or intracolonic administration (■) at 3 mg kg^{-1}, n = 3.

Fig. 20 compares two Upjohn renin inhibitors (Burton et al 1992), the difference between the two structures being that peptide **XII** contains a methyl group on an interior amide group. This peptide displays twice the rate of intestinal permeability of peptide **XI**.

Unfortunately, not all modifications to amide bonds may be beneficial to receptor affinity or in-vitro potency. In the series of GPIIb/IIIa antagonists (Ali et al 1994) related to SK&F 106760 (Table 4), *N*-methylation of the alpha amine of arginine gave an analogue with enhanced potency, but *N*-methylation of the α-amine of either Gly or Asp gave analogues with diminished potency. It became clear that a template needed to be found that was more amenable to further reductions in hydrophilicity, or possibly the number of potential hydrogen bonds.

Replacement of the peptidic template with a nonpeptide template: development of orally active GPIIb/IIIa antagonists

Another strategy was taken at SmithKline Beecham in the pursuit of orally active GPIIb/IIIa antagonists: to search for nonpeptide templates that retained the high affinity of SK&F 107260, but were more amenable to modifications that could enhance oral activity. Success in such a search had yet to be described in the literature. This search required the development of an understanding of the receptor-bound conformation of the peptides SK&F 106760 and SK&F 107260, bearing the global constraint of a cyclic scaffold, to see if this structural information could serve as a pharmacophore model (Fig. 1), from which nonpeptide leads could be designed.

Development of a pharmacophore model of peptidic GPIIb/IIIa antagonists

The conformations of SK&F 106760 and 107260 were examined by ^{1}H-NMR spectroscopy in methanol (Kopple et al 1992). With SK&F 107260, two discrete sets of NMR signals and many assignable NOEs were observed. This

	Caco-2 cell permeability ($cm\ s^{-1} \times 10^6$)	Log partition coefficient	Number of potential hydrogen bonds
I Ac-Phe-Phe-Phe-NH_2	0.67	2.30	9
II Ac-Phe-Phe-(N$^{\alpha}$Me)Phe-NH_2	2.88	2.63	8
III Ac-Phe-(N$^{\alpha}$Me)Phe-(N$^{\alpha}$Me)Phe-NH_2	6.11	2.53	7
IV Ac-(N$^{\alpha}$Me)Phe-(N$^{\alpha}$Me)Phe-(N$^{\alpha}$Me)Phe-NH_2	16.7	2.92	6
V Ac-(N$^{\alpha}$Me)Phe-(N$^{\alpha}$Me)Phe-(N$^{\alpha}$Me)Phe-NH-Me	33.9	3.24	5

FIG. 19. Series of peptides bearing increasing numbers of *N*-methylated amides (Conradi et al 1991).

information was employed in a constrained distance geometry study of SK&F 107260. By this study, the two sets of signals in the NMR spectrum were related to two low-energy conformations (Fig. 21) that were quite similar in all but the orientation of the amide bond between aspartic acid and the C-terminal aniline. In the major NMR component that amide bond gave rise to a γ-turn about aspartic acid. In the minor NMR component that amide bond gave rise to a helical turn about aspartic acid. In both conformations there was a turn about arginine and a fully extended glycine. Crystals were ultimately obtained from a sample of the nitrate salt of SK&F 107260, from which an X-ray crystal structure was obtained. The crystal structure was identical to the minor component in the NMR. Fig. 22 (Kopple et al 1992) displays an ORTEP model of the X-ray structure, showing once again, the turn-extended-turn conformation about the RGD portion of SK&F 107260.

SK&F 106760 proved to be more flexible than SK&F 107260 in solution, as determined again by NMR spectroscopy and distance geometry (Kopple et al 1992). This greater flexibility arose presumably from the greater degrees of freedom in the disulphide tether of SK&F 106760. The

XI P = 0.9 cm s^{-1} x 10^6 XII P = 1.7 cm s^{-1} x 10^6

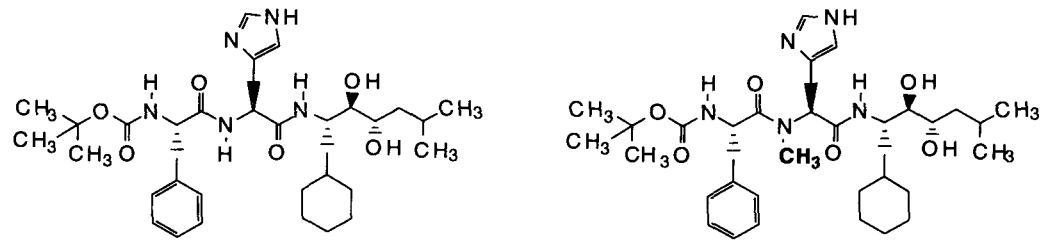
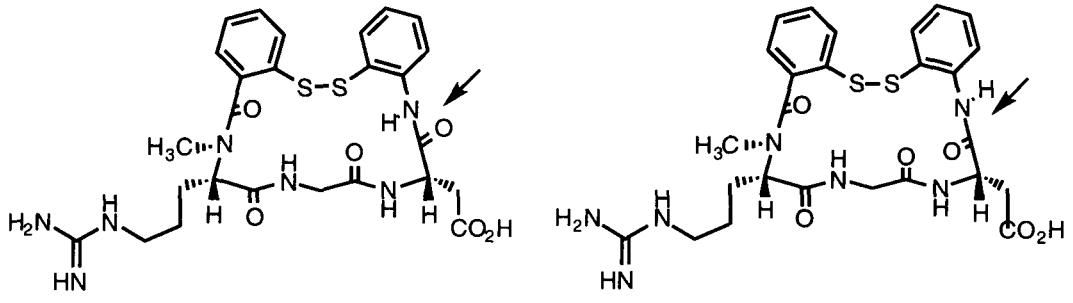


FIG. 20. Renin inhibitor containing an *N*-methyl amide shows increased intestinal permeability (Burton et al 1992).



most probable conformation of major component in methanol at 203 K

X-ray crystal structure and probable conformation of minor component in methanol at 203 K

FIG. 21. Conformations of SK&F 107260 (Kopple et al 1992).

NOEs obtained from the NMR were employed again in a distance geometry study of SK&F 106760. Despite a greater flexibility, a turn-extended-turn conformation was identified again (Fig. 23), that was comparable with the major component conformation for SK&F 107260.

SK&F 106760 and SK&F 107260 were but two peptides in a family of RGD-containing peptides constrained in cyclic structures (Bean et al 1992; Peishoff et al 1992). Comparison of the conformational structures of these cyclic peptides with their antiaggregatory activities and affinities for GPIIb/IIIa led us to adopt the dominant conformation of SK&F 107260 as a pharmacophore model of an RGD peptide bound to GPIIb/IIIa (Peishoff et al 1992; Samanen et al 1994). The model was also further examined through the synthesis, conformational evaluation and bioassay of linear analogues of SK&F 107260 containing replacements for both arginine and aspartic acid as regional conformational constraints (Fig. 1), that mimicked the turns about both residues in SK&F 107260 (Callahan et al 1992). In both cases the turn mimetic peptides were as potent as the linear peptide but less active than SK&F 107260, suggesting that single-turn mimetics provided an insufficient amount of conformational constraint. Nonpeptide structures that contained multiple constraints were sought, therefore, with the hope that these nonpeptide structures would serve as templates in the design of GPIIb/IIIa antagonists with oral activity.

Table 4. *N*-Methylated analogues of SK&F 106760 (Ali et al 1994).

	Platelet aggregation IC50 (μM)
Ac-cyclo(Cys-Arg-Gly-Asp-Pen)-NH_2	4·12
Ac-cyclo(Cys-(NαCH_3)Arg-Gly-Asp-Pen)-NH_2	0·36
Ac-cyclo(Cys-Arg-(NαCH_3)Gly-Asp-Pen)-NH_2	73·4
Ac-cyclo(Cys-Arg-Gly-(NαCH_3)Asp-Pen)-NH_2	136·7

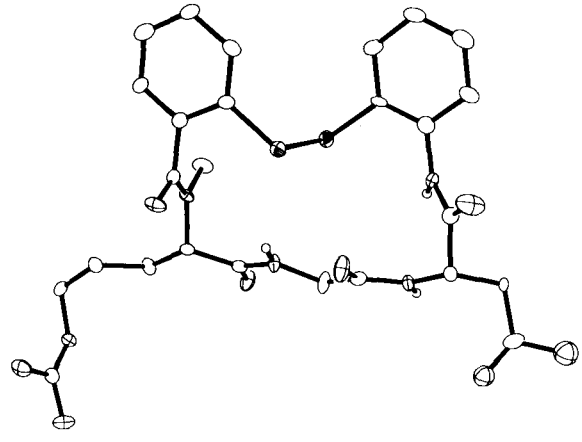


FIG. 22. The X-ray crystal structure of SK&F 107260 (Kopple et al 1992).

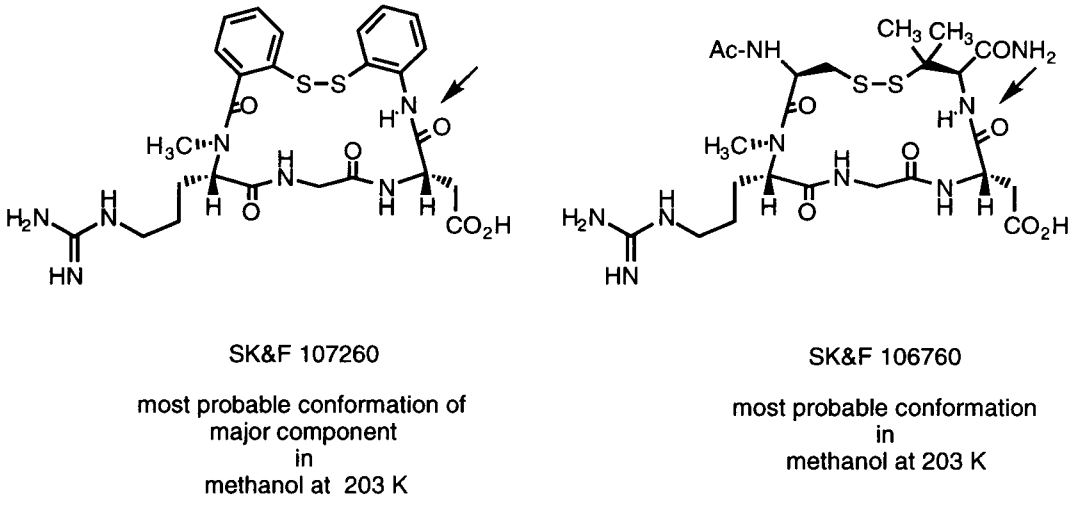


FIG. 23. Comparison of the conformations of SK&F 107260 with SK&F 106760 (Kopple et al 1992).

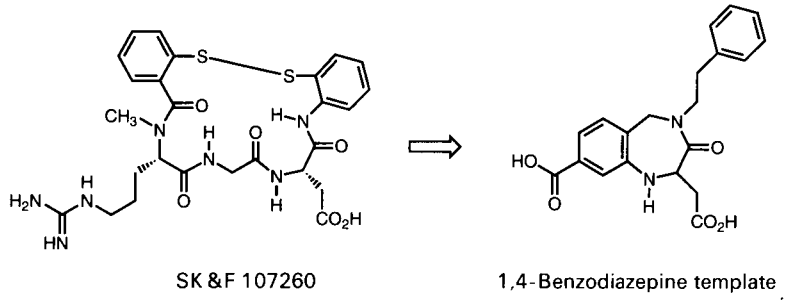


FIG. 24. Structures of SK&F 107260 and 1,4-benzodiazepine template.

Direct design of a 1,4-benzodiazepine GPIIb/IIIa antagonist, SB 207448, from the peptide pharmacophore model. 1,4-Benzodiazepines were found to be attractive as a potential Gly-Asp mimetic (Ku et al 1993). The 1,4-diazepine ring was ideally suited as a mimetic of the γ turn about aspartic acid in SK&F 107260, and the benzo group could serve as a mimetic of the fully extended glycine (Fig. 24). The most potent globally constrained peptide analogue of SK&F 106760, contained a chiral β-phenylcysteine (Fig. 25) (Ali et al 1994).

This compound suggested the incorporation of a phenethyl group in position four of the Gly-Asp mimetic.

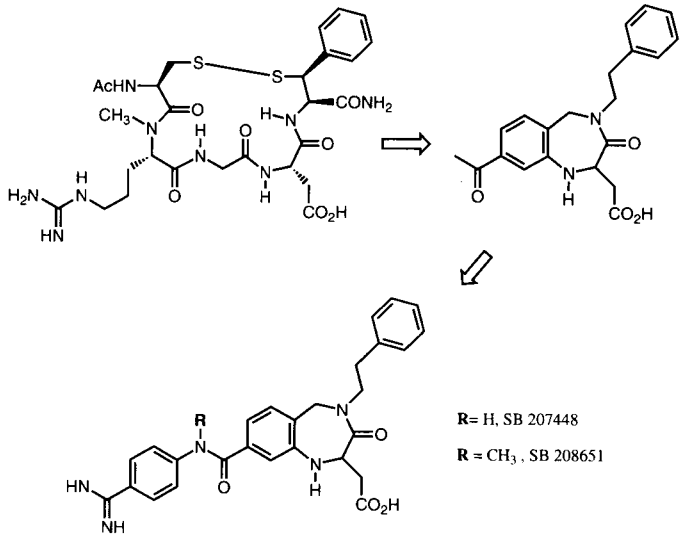


FIG. 25. Relationship of the penicillamine sidechain of SK&F 109064 which relate to the 4-phenethyl group in the 1,4-benzodiazepine template and resulting compounds SB 207448 and SB 208651.

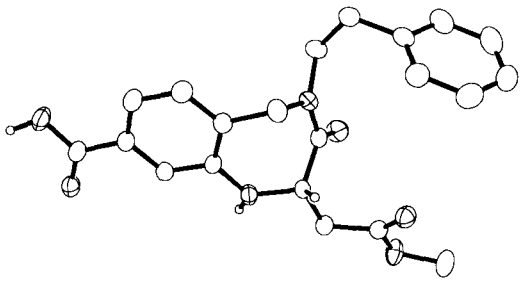


FIG. 26. X-Ray crystal structure of the 1,4-benzodiazepine precursor (Bondinell et al 1994).

Synthesis of this compound afforded crystalline material that allowed the determination of an X-ray crystal structure, which is displayed in Fig. 26 (Ku et al 1993) showing only the *S*-enantiomer. Overlay of the model from the crystal structure of the benzodiazepine precursor onto SK&F 107260 shows a very high degree of correspondence between overlayed atoms (Fig. 27). At this point in our research, other laboratories had described the employment of phenyl amidines as mimetics for the arginine side chain. Borrowing on this information, the Gly-Asp mimetic design was completed with a phenyl amidine to provide yet another degree of constraint (Fig. 25).

Table 5 displays the in-vitro activities for the Gly-Asp mimetic SB 207448 (Ku et al 1993; Bondinell et al 1994). SB 207448 displays affinity and potency that is comparable with SK&F 107260. As a bonus, the nonpeptide displays high selectivity against $\alpha v\beta 3$, the integrin with the greatest similarity to GPIIb/IIIa. The compound was found to have potent antiaggregatory activity in conscious dogs. Ex-vivo platelet aggregation was completely inhibited after a short infusion of 0.3 mg kg^{-1} SB 207448 (Fig. 28) (Bondinell et al 1994). The duration is comparable with SK&F 107260. Unfortunately, a short infusion of 3 mg kg^{-1} SB 207448 by intraduodenal administration, produced no inhibition. Encouraged by the intravenous result, the synthesis of further series of analogues of 1,4-benzodiazepines continued.

Discovery of SB 208651, the first 1,4-benzodiazepine GPIIb/IIIa antagonist with oral activity. Among the first analogues that were prepared was an analogue bearing a methyl on the tethering amide, SB 208651 (Fig. 25) (Bondinell et al 1994). The in-vitro activities of SB 208651, shown in Table 6, were comparable with SB 207448. The 1,4-benzodiazepine precursor to SB 208651 has been resolved by chiral HPLC. The absolute configuration of the *R*-enantiomer was obtained by X-ray crystal structure of a *p*-bromobenzoyl analogue of the (+)-enantiomer. Consequently, both the *R*- and *S*-enantiomers of SB 208651 could be prepared, and all of the activity of SB 208651 was found to reside in the *S*-enantiomer, which is the identical situation in the peptides (data not shown) (Bondinell et al 1994).

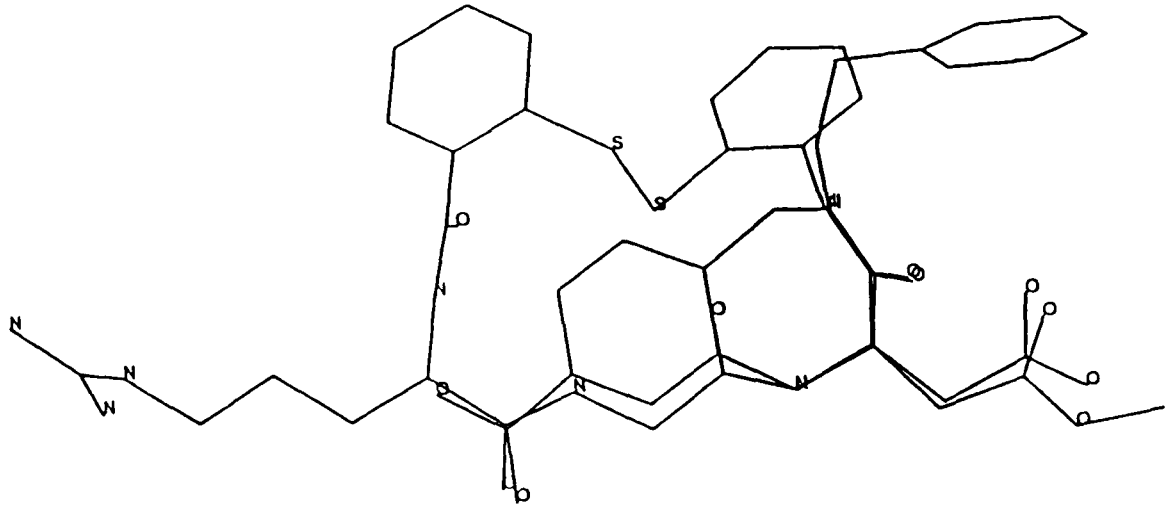


FIG. 27. Overlay of the X-ray crystal structure of the 1,4-benzodiazepine precursor (Ku et al 1993) with SK&F 107260.

Table 5. In-vitro activity of SK&F 107260 and SB 207448 (Bondinell et al 1994).

	Anti-aggregation	hGPIIb/IIIa[b] vs	Binding inhibition hGPIIb/IIIa[c] vs	$\alpha_v\beta_3$[d] vs
	hPRP/ADP[a] IC50 (nM)	Biotinylated Fg K_i (nM)	[^{3}H]SK&F 107260 K_i (nM)	[^{3}H]SK&F 107260 K_i (nM)
SK&F 107260	57.0	0.62	2.08	2.86
SB 207448	150	1.5	2.8	17.0

[a] Inhibition of platelet aggregation induced by ADP in human platelet-rich plasma. [b]Inhibition of biotinylated fibrinogen binding to GPIIb/IIIa purified from human platelets, immobilized on microtitre plates (ELISA). [c]Inhibition of [^{3}H]SK&F 107260 binding to GPIIb/IIIa purified from human platelets, reconstituted in liposomes. [d]Inhibition of [^{3}H]SK&F 107260 binding to $\alpha_v\beta_3$ purified from human platelets, immobilized on microtitre plates.

Table 6. In-vitro biological activity of SB 207448 and SB 208651 (Bondinell 1994).

	Anti-aggregation	hGPIIb/IIIa[b] vs	Binding inhibition hGPIIb/III[c] vs	$\alpha_v\beta_3$[d] vs
	hPRP/ADP[a] IC50 (nM)	Biotinylated Fg K_i (nM)	[^{3}H]SK&F 107260 K_i (nM)	[^{3}H]SK&F 107260 K_i (nM)
SB 207448	150	1.5	2.8	17.0
SB 208651	65	1.4	1.6	28.0

[a] Inhibition of platelet aggregation induced by ADP in human platelet-rich plasma. [b]Inhibition of biotinylated fibrinogen binding to GPIIb/IIIa purified from human platelets, immobilized on microtitre plates (ELISA). [c]Inhibition of [^{3}H]SK&F 107260 binding to GPIIb/IIIa purified from human platelets, reconstituted on liposomes. [d]Inhibition of [^{3}H]SK&F 107260 binding to $\alpha_v\beta_3$ purified from human platelets, immobilized on microtitre plates.

As with SB 207448, intravenous administration of SB 208651 gave rapid inhibition of ex-vivo platelet aggregation in conscious dogs (Fig. 29) (Bondinell et al 1994). In contrast, however, intraduodenal administration of SB 208651 gave a rapid inhibition of ex-vivo platelet aggregation, with a duration comparable with the intravenous dose. Since the effects from intraduodenal administration of a 3 mg kg^{-1} dose were nearly identical to intravenous administration of a 0.3 mg kg^{-1} dose, the apparent bioavailability of SB 208651 is estimated to be around 10%. The intestinal permeabilities of both compounds were determined (Table 7). Intriguingly, no intestinal absorption was observed with SB 207448. The intestinal permeability of SB 208651, however, was comparable with mannitol which displays an oral bioavailability in man of about 20%. An explanation for this dramatic enhancement in intestinal absorption of SB 208651 over SB 207448 may be that methylation of an amide bond in SB 207448 increased intestinal permeability due to a decrease in the number of potential hydrogen bonds. This increased intestinal permeability may arise not from an increase in lipophilicity, but from a decrease in the desolvation energy (Karls et al 1991) required to remove a hydrophilic molecule from the aqueous environment of the lumen into the lipophilic environment of the intestinal epithelial cell membrane.

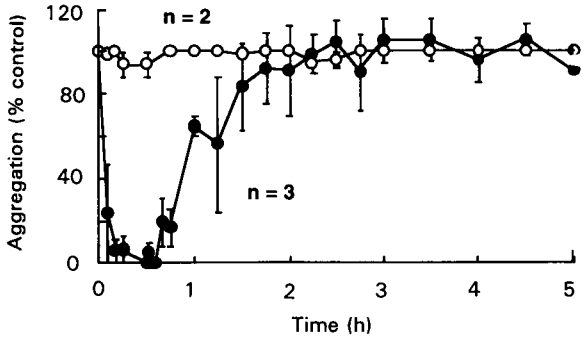


FIG. 28. Inhibition of ex-vivo platelet aggregation (Nichols et al 1994) by SB 207448 after intravenous (●, 0.3 mg kg^{-1}) or intraduodenal (○, 3 mg kg^{-1}) administration (Bondinell et al 1994).

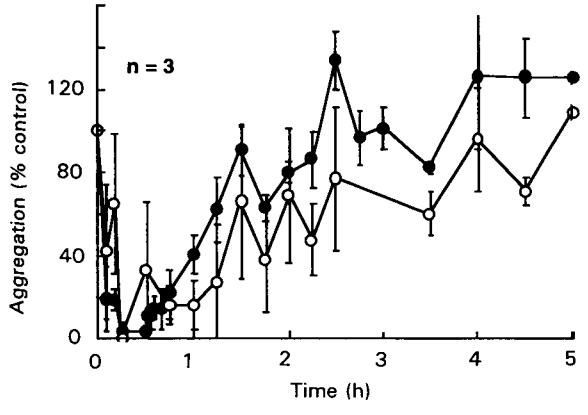


FIG. 29. Inhibition of ex-vivo platelet aggregation (Nichols et al 1994) by SB 208651 after intravenous (●, 0.3 mg kg^{-1}) or intraduodenal (○, 3 mg kg^{-1}) administration (Bondinell et al 1994).

Summary

In this review it has been shown that in-vitro assays have been developed that provide meaningful, reliable intestinal permeability data that can help in analogue design, and in the selection of compounds for oral bioavailability assay. In-vivo assays have been developed that can provide meaningful, reliable intestinal bioavailability data that can help in the selection of a clinical candidate.

Extensive studies of RGD peptides containing both global and regional conformational constraints (Fig. 1) support the turn-extended-turn conformation as a receptor-bound conformation of RGD peptides at the platelet fibrinogen receptor GPIIb/IIIa. This conformation has been reported subsequently in other cyclic RGD peptides by researchers at Glaxo (Hann et al 1992) and at DuPont Merck (Bach et al 1994). This model conformation enabled the design of a potent and orally active nonpeptide fibrinogen receptor antagonist. With SB 207448 and SB 208651, it has been shown that nonpeptide ligands can be designed from pharmacophore models based upon the structures of highly constrained peptides.

Table 7. Intestinal permeabilities (cm h^{-1}) of SB 207448 and SB 208651.

Compound	P_{ileum}
SB 207448	<0·0001
SB 208651	0·0120
SK&F 107260	0·003
Mannitol	0·0192
Diazepam	0·133

Acknowledgements

The authors wish to express their appreciation to Giora Feuerstein, John Gleason, William Huffman, Brian Metcalf and Robert Ruffolo for their support of these studies.

References

Ali, F. E., Bennett, D. B., Calvo, R. R., Elliott, J. D., Hwang, S.-M., Ku, T. W., Lago, M. A., Nichols, A. J., Romoff, T. T., Shah, D. H., Vasko, J. A., Wong, A. S., Yellin, T. O., Yuan, C.-K., Samanen, J. M. (1994) Conformationally constrained peptides and semipeptides derived from RGD as potent inhibitors of the platelet fibrinogen receptor and platelet aggregation. J. Med. Chem. 37: 769–780

Ali, F. E., Samanen, J. M., Calvo, R., Romoff, T., Yellin, T., Vasko, J., Powers, D., Stadel, J., Bennett, D., Berry, D., Nichols, A. (1992) Potent fibrinogen receptor antagonists bearing conformational constraints. In: Smith, J. A., Rivier, J. E. (eds) Proteins: Chemistry, Structure and Biology, Proc. 13th American Peptide Symposium. Escom Science Publishers, Leiden 761–762

Andrieux, A., Hudry-Clergeon, G., Ryckewaert, J.-J., Chapel, A., Ginsberg, M. H., Plow, E. F., Marguerie, G. (1989) Amino acid sequences in fibrinogen mediating its interaction with its platelet receptor, GPIIb/IIIa. J. Biol. Chem. 264: 9258–9265

Audus, K. L., Bartel, R. L., Hidalgo, I. J., Borchardt, R. T. (1990) The use of cultured epithelial and endothelial cells for drug transport and metabolism studies. Pharm. Res. 7: 435–451

Bach, A. C. I., Eyermann, C. J., Gross, J. D., Bower, M. J., Harlow, R. L., Weber, P. C., DeGrado, W. F. (1994) Structural studies of a family of high affinity ligands for GPIIb/IIIa. J. Am. Chem. Soc. 116: 3207–3219

Bean, J. W., Kopple, K. D., Peishoff, C. E. (1992) Conformational analysis of cyclic hexapeptides containing the D-Pro-L-Pro sequence to fix β-turn positions. J. Am. Chem. Soc. 114: 5328–5334

Bondinell, W. E., Keenan, R. M., Miller, W. H., Ali, F. E., Allen, A. C., DeBrosse, C. W., Eggleston, D. S., Erhard, K. F., Haltiwanger, R. C., Huffman, W. F., Hwang, S.-M., Jakas, D. R., Koster, P. F., Ku, T. W., Lee, C. P., Nichols, A. J., Ross, S. T., Samanen, J. M., Valocik, R. E., Vasko-Moser, J. A., Venslavsky, J. W., Wong, A. S., Yuan, C.-K. (1994) Design of a potent and orally active nonpeptide platelet fibrinogen receptor (GPIIB/IIIA) antagonist. Bioorg. Med. Chem. 2: 897–908

Bowers, C. Y., Reynolds, G. A., Chang, D., Hong, A., Chang, K., Momany, F. A. (1980) A study of the regulation of growth hormone release from the pituitaries of rats in vitro. Endocrinology 108: 1071–1080

Burton, P. S., Conradi, R. A., Hilgers, A. R., Ho, N. F. H., Maggiora, L. L. (1992) The relationship between peptide structure and transport across epithelial cell monolayers. J. Contr. Rel. 19: 87–98

Burton, P. S., Conradi, R. A., Hilgers, A. R., Ho, N. F. H. (1993) Evidence for a polarized efflux system for peptides in the apical membrane of Caco-2 cells. Biochem. Biophys. Res. Commun. 190: 760–766

Callahan, J. F., Bean, J. W., Burgess, J. L., Eggleston, D. S., Hwang, S. M., Kopple, K., Koster, P. F., Nichols, A., Peishoff, C., Samanen, J., Vasko, J. A., Wong, A., Huffman, W. (1992) Design and Synthesis of a C7 mimetic for the predicted γ-turn conformation found in several constrained RGD antagonists. J. Med. Chem. 35: 3970–3972

Callahan, J. F., Newlander, K. A., Burgess, J. L., Eggleston, D., Nichols, A., Wong, A., Huffman, W. F. (1993) The use of γ-turn mimetics to define peptide secondary structure. Tetrahedron 49: 3479–3488

Conradi, R. A., Hilgers, A. R., Ho, N. F. H., Burton, P. S. (1991) The influence of peptide structure on transport across Caco-2 cells. Pharm. Res. 8: 1453–1459

Conradi, R. A., Hilgers, A. R., Ho, N. F. H., Burton, P.S. (1992) The influence of peptide structure on transport across Caco-2 cells. II. Peptide bond modification which results in improved permeability. Pharm. Res. 9: 435–439

Constantinides, P. P., Scalart, J., Lancaster, C., Marcello, J., Marks, G., Ellens, H., Smith, P. L. (1994) Formulation and intestinal absorption enhancement evaluation of water-in-oil microemulsions incorporating medium-chain glycerides. Pharm. Res. 11: 1385–1390

Dantzig, A. H., Bergin, L. (1988) Carrier-mediated uptake of cephalexin in human intestinal cells. Biochem. Biophys. Res. Commun. 155: 1082–1087

Dantzig, A. H., Bergin, L. (1990) Uptake of the cephalosporin cephalexin, by a dipeptide transport carrier in the human intestinal cell, Caco-2. Biochim. Biophys. Acta 1027: 211–217

Ganapathy, V., Leibach, F. H. (1985) Is intestinal peptide transport energized by a proton gradient? Am. J. Physiol. 249: G153–G160

Gartner, T. K., Bennett, J. S. (1985) The tetrapeptide analogue of the cell attachment site of fibronectin inhibits platelet aggregation and fibrinogen binding to activated platelets. J. Biol. Chem. 260: 11891–11894

Ginsberg, M. H., Pierschbacher, M. D., Ruoslahti, E., Marguerie, G. A., Plow, E. F. (1985) Inhibition of fibronectin binding to platelets by proteolytic fragments and synthetic peptides which support fibroblast adhesion. J. Biol. Chem. 260: 3931–3936

Gochoco, C. H., Ryan, F. M., Miller, J., Smith, P. L., Hidalgo, I. J. (1994) Uptake and transepithelial transport of the orally-absorbed cephalosporin, cephalexin, in human intestinal cell line, Caco-2. Int. J. Pharm. 104: 187–202

Gunby, P. (1992) Cardiovascular diseases remain nation's leading cause of death. J. Am. Med. Assoc. 267: 335–336

Hann, M. M., Carter, B., Kitchin, J., Ward, P., Pipe, A., Broomhead, J., Hornby, E., Forster, M., Perry, C. (1992) An investigation of the bioactive conformation of Arg-Gly-Asp containing cyclic peptides and snake venom peptides which inhibit human platelet aggregation. In: Roberts, S. M. (ed.) Molecular Recognition: Chemical and Biochemical Problems II. The Royal Society of Chemistry, Cambridge, pp 145–159

Hashimoto, N., Fujioka, T., Hayashi, K., Odaguchi, K., Toyoda, T., Nakamura, M., Hirano, K. (1994) Renin inhibitor, relationship between molecular structure and oral absorption. Pharm. Res. 11: 1443–1447

Hidalgo, I. J., Raub, T. J., Borchardt, R. T. (1989) Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 96: 736–749

Hidalgo, I. H., Ryan, F. M., Marks, G. J., Smith, P. L. (1993) pH-Dependent transcellular transport of cephalexin in rabbit intestinal mucosa. Int. J. Pharm. 98: 83–92

Hidalgo, I. J., Bhatnagar, P., Lee, C., Miller, J., Cucullino, G., Smith, P. L. (1995) Structural requirements for interaction with the oligopeptide transporter in Caco-2 cells. Pharm. Res. 12: 317–319

Holzemann, G. (1991) Peptide conformation mimetics. Part 1. Kontakte (Darmstadt) 1: 3–12

Horwell, D. C., Birchmore, B., Boden, P. R., Higginbottom, M., Ping Ho, Y., Hughes, J., Hunter, J. C , Richardson, R. S. (1990) α-Methyl tryptophanylphenylalanines and their aryl ethylamine "dipeptoid" analogues of the tetrapeptide cholecystokinin (30-33). Eur. J. Med. Chem. 25: 53–60

Hunter, J., Hirst, B. H., Simmons, N. L. (1991) Transepithelial vinblastine secretion mediated by P-glycoprotein is inhibited by forskolin derivatives. Biochem. Biophys. Res. Commun. 181: 671–676

Jung, G., Beck-Sickinger, A. G. (1992) Multiple peptide synthesis methods and their applications. Angew. Chem. Int. Ed. Engl. 31: 367–486

Karls, M. S., Rush, B. D., Wilkinson, K. F., Vidmar, T. J., Burton, P. S., Ruwart, M. J. (1991) Desolvation energy: a major determinant of absorption, but not clearance, of peptides in rats. Pharm. Res. 8: 1477–1481

Kleeman, H. W., Heitsch, H., Henning, R., Kramer, W., Kocher, U., Lerch, U., Linz, W., Nickel, W. U., Ruppert, D., Urbach, H., Utz, R., Wagner, A., Weck, R., Wiegand, F. (1992) Renin inhibitory pentols showing improved enteral bioavailability. J. Med. Chem. 35: 559–567

Kloczewiak, M., Timmons, S., Bednarek, M. A., Sakon, M., Hawiger, J. (1989) Platelet receptor recognition domain on the γ chain of human fibrinogen and its synthetic peptide analogues. Biochemistry 28: 2915–2919

Kloczewiak, M., Timmons, S., Lukas, T. J., Hawiger, J. (1984) Recognition site for the platelet receptor is present on the 15-residue fragment of the γ chain of human fibrinogen and is not involved in the fibrin polymerization reaction. Biochemistry 23: 1767–1774

Kopple, K. D., Baures, P. W., Bean, J. W., D'Ambrosio, C. A., Hughes, J. L., Peishoff, C. E., Eggleston, D. S. (1992) Conformations of Arg-Gly-Asp containing heterodetic cyclic peptides: solution and crystal studies. J. Am. Chem. Soc. 114: 9615–9623

Ku, T. W., Ali, F. E., Barton, L. S., Bean, J. W., Bondinell, W. E., Burgess, J. L., Callahan, J. F., Calvo, R. R., Chen, L., Eggleston, D. S., Gleason, J. G., Huffman, W. F., Hwang, S. M., Jakas, D. R., Karash, C. B., Keenan, R. M., Kopple, K. D., Miller, W. M., Newlander, K. A., Nichols, A., Parker, M. F., Peishoff, C. E., Samanen, J. M., Uzinskas, I., Venslavsky, J. W. (1993) Direct design of a potent non-peptide fibrinogen receptor antagonist based on the structure and conformation of a highly constrained cyclic RGD peptide. J. Am. Chem. Soc. 115: 8861–8862

Laker, M. F., Bull, H. J., Menzies, I. S. (1982) Evaluation of mannitol for use as a probe marker of gastrointestinal permeability in man. Eur. J. Clin. Invest. 12: 485–491

Marks, G. I., Ryan, F. M., Hidalgo, I. J., Smith, P. L. (1991) Mannitol, as a marker for intestinal integrity in in vitro absorption studies. Gastroenterology 100: A697

Matuszewska, B., Liversidge, G. G., Ryan, F., Dent, J., Smith, P. L. (1988) In vitro study of intestinal absorption and metabolism of 8-L-argenine vasopressin and its analogues. Int. J. Pharm. 46: 111–120

Meunier, L. D., Kissinger, J. T., Marcello, J., Nichols, A. J., Smith, P. L. (1993) A chronic access model for direct delivery of drugs into the intestine of conscious dogs. J. Lab. Animal Sci. 43: 466–470

Nichols, A. J., Ruffolo, R. R., Huffman, W. F., Poste, G., Samanen, J. (1992) Development of GpIIb/IIIa Antagonist as antithrombotic drugs. Trends Pharmacol. Sci. 13: 413–417

Nichols, A. J., Vasko, F. A., Koster, P. F., Valocik, R. E., Samanen, J. M. (1994a) GPIIb/IIIa Antagonists as novel antithrombotic drugs: potential therapeutic agents. In: Metcalf, B.W., Dalton, B. J., Poste, G. (eds) Cellular Adhesion: Molecular Definition to Therapeutic Potential. Plenum Press, New York, pp 213–237

Nichols, A. J., Vasko, J. A., Koster, P. F., Valocik, R. E., Rhodes, G. R., Miller-Stein, C., Boppana, V., Samanen, J. M. (1994b) The in vivo pharmacological profile of the novel glycoprotein IIb/IIIa antagonist, SK&F 106760. J. Pharm. Exp. Ther. 270: 614–621

Peishoff, C. E., Ali, F. E., Bean, J. W., Calvo, R., D'Ambrosio, C. A., Eggleston, D. S., Kline, T. P., Koster, P. F., Nichols, A., Powers, D., Romoff, T., Samanen, J. M., Stadel, J., Vasko, J. A., Wong, A., Kopple, K. D. (1992) Investigation of conformational specificity at GPIIb/IIIa: evaluation of conformationally constrained RGD peptide analogs. J. Med. Chem. 35: 3962–3969

Plow, E. F., Pierschbacher, M. D., Ruoslahti, E., Marguerie, G., Ginsberg, M. H. (1987) Arginyl-glycyl-aspartic acid sequences and fibrinogen binding to platelets. Blood : 110–115

Rosenberg, S. H., Spina, K. P., Condon, S. L., Polakowski, J., Yao, Z., Kovar, P., Stein, H. H., Cohen, J., Barlow, J. L., Klinghofer, V., Egan, D. A., Tricarico, K. A., Perrun, T. J., Baker, W. R., Kleinert, H. D. (1993b) Studies directed toward the design of orally active renin inhibitors. J. Med. Chem. 36: 460–467

Rosenberg, S. H., Spina, K. P., Woods, K. W., Polakowski, J., Martin, D. L., Yao, Z., Stein, H. H., Cohen, J., Barlow, J. L., Egan, D. A., Tricarico, K. A., Baker, W. R., Kleinert, H. D. (1993a) Studies directed toward the design of orally active renin inhibitors. J. Med. Chem. 36: 449–459

Saffran, M., Kumar, G. S., Savariar, C., Burnham, J. C., Williams, F., Neckers, D. C. (1986) A new approach to the oral administration of insulin and other peptide drugs. Science 233: 1081–1084

Samanen, J. (1985) Biomedical polypeptides—A wellspring of pharmaceuticals. In: Gebelein, C. G, Carraher, C. E. J. (eds) Bioactive Polymeric Systems, An Overview . Plenum Press, New York. pp 279–382

Samanen, J., Ali, F. E., Romoff, T., Calvo, R., Sorenson, E., Vasko, J., Berry, D., Bennett, D., Strohsacker, M., Powers, D., J., S., Nichols, A. (1991) Development of an RGD-peptide fibrinogen receptor antagonist with potent antiaggregatory activity in vitro. J. Med. Chem. 34: 3114–3125

Samanen, J., Weinstock, J., Hempel, J., Keenan, R. M., Hill, D. T., Ohlstein, E. H., Weidley, E. F., Aiyar, N., Edwards, R. (1992) A molecular model of angiotensin II (Ang II) for rational design of small Ang II antagonists with increased potency. In: Riviere, J. E., Smith, J. A. (eds) Peptides: Structure and Biology; Proceedings of the 12th American Peptide Symposium. Leiden: ESCOM Science Publishers, pp 386–388

Samanen, J. M., Peishoff, C. E., Keenan, R. M., Weinstock, J. M. (1993) Refinement of a molecular model of angiotensin II (AII) employed in the discovery of potent nonpeptide antagonists. Bioorg. Med. Chem. Lett. 3: 909–914

Samanen, J., Ali, F., Bean, J., Callahan, J., Huffman, W., Kopple, K., Peishoff, C , Nichols, A. (1994) Peptide mimetics as adhesion molecule antagonists. In: Metcalf, B. W., Dalton, B. J., Poste, G. (eds) Cellular Adhesion: Molecular Definition to Therapeutic Potential. Plenum Press, New York pp 259–290

Smith, J. W. (1994) The structural basis of integrin-ligand (RGD) interaction. In: Cheresh, D. A., Mecham, R. P. (ed.) Integrins, Molecular and Biological Response to the Extracellular Matrix. Academic Press, New York, pp 1–32

Smith, P. L. (1995) Intestinal absorption and secretion: in vivo studies. In: Gaginella, T. S. (ed.) Methods in Gastrointestinal Pharmacology. CRC, New York. pp 85–99

Smith, P. L., Mirabelli, C., Fondacaro, J., Ryan, F., Dent, J. (1988) Intestinal 5-fluorouracil absorption: use of Ussing chambers to assess transport and metabolism. Pharm. Res. 5: 598–603

Smith, P. L., Wall, D. A., Gochoco, C. H., Wilson, G. Routes of Delivery: Case Studies; (5) (1992) Oral absorption of peptides and proteins. Advanced Drug Delivery Reviews 8: 253–290

Smith, P. L., Eddy, E. P., Lee, C., Wilson, G. (1993) Exploitation of the intestinal oligopeptide transporter to enhance drug absorption. Drug Del. 1: 103–111

Smith, P. L., Yeulet, S. E., Citerone, D. R., Drake, F., Cook, M., Wall, D. A., Marcello, J. (1994) SK&F 110679: Comparison of absorption following oral or respiratory administration. J. Contr. Rel. 28: 67–77

Swaan, P. W., Marks, G. J., Ryan, F. M., Smith, P. L. (1994) Determination of transport rates for arginine and acetaminophen in rabbit intestinal tissues in-vitro. Pharm. Res. 11: 283–287

Thwaites, D. T., Brown, C. D. H., Hirst, B. H., Simmons, N. L. (1993) H^+-coupled dipeptide (glycylsarcosine) transport across apical and basal membranes of human intestinal Caco-2 cell monolayers display distinct characteristics. Biochim. Biophys. Acta 1151: 237–245

Toniolo, C. (1989) Structure of conformationally constrained peptides: from model compounds to bioactive peptides. Biopolymers 28: 247–257

Toniolo, C. (1990) Conformationally restricted peptides through short-range cyclizations. Int. J. Pept. Protein Res. 35: 287–300

Ussing, H. H., Zerahn, K. (1951) Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta Physiol. Scand. 23: 110–127

Walker, R. F., Codd, E. H., Barone, F. C., Nelson, A. H., Goodwin, T., Campbell, S. A. (1990) Oral activity of the growth hormone releasing peptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 in rats, dogs and monkeys. Life Sci. 47: 29–36

Wilson, G., Hasson, I. F., Dix, C. J., Williamson, I., Shah, R., Mackay, M., Arturssou, P. (1990) Transport and permeability properties of human Caco-2 cells: an in vitro model of the intestinal epithelial cell barrier. J. Contr. Rel. 11: 25–40

Yeh, P.-Y., Smith, P. L., Ellens, H. (1994) Effect of medium-chain glycerides on physiological properties of rabbit intestinal epithelium in vitro. Pharm. Res. 11: 1148–1154