International Journal of Pharmaceutics, 70 (1991) 271–283
© 1991 Elsevier Science Publishers B.V. 0378-5173/91/$03.50
ADONIS 0378517391001714

IJP 02378

Comparison of enzyme activities of tissues lining portals of absorption of drugs: Species differences

Xin Hua Zhou and Alain Li Wan Po

Drug Delivery Research Group, School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL (U.K.)

(Received 10 September 1990)
(Accepted 22 December 1990)

Key words: Aminopeptidase; Cholesterol esterase; Peptide delivery; Prodrug delivery; Species differences

Summary

The aminopeptidase and esterase activities of dermal, nasal, buccal, rectal and intestinal tissues from the rat, rabbit, guinea-pig and dog were investigated. The results demonstrate that dermal, nasal and buccal tissues show the same species ranking with respect to enzymic activities. For those tissues, the species ranking for both crude and protein-normalised aminopeptidase activity was found to be dog < rat < guinea-pig < rabbit. With respect to esterase activity of those tissues the relative ranking was guinea-pig < rat < rabbit. Rectal and intestinal tissues showed a different ranking order to the other tissues. For aminopeptidase activity, the ranking was rat < dog < rabbit < guinea-pig. For the esterase activity of intestinal tissue, the rank order was guinea-pig < rat < rabbit. Intestinal tissues consistently showed the highest enzymic activities across all species studied, suggesting that enzyme inhibition may be necessary for effective delivery of poorly absorbed peptide or esterified drugs via this route. The dermal route presents the least peptidase activity.

Introduction

An increasing number of peptide and protein drugs are being introduced into therapeutics as a result of recent developments in biotechnology. Examples include analogues of luteinizing hormone releasing hormone (Anik, 1984; Sandow and Petrie, 1985) and cyclosporin (Wood et al., 1983). While a number of those drugs have satisfactory bioavailabilities, the majority of peptide and protein drugs are poorly absorbed into the systemic circulation when administered by non-parenteral routes due to first-pass metabolism or poor permeabilities across the absorption barriers. Therefore over recent years, there has been a surge in research activity directed towards improving the non-parenteral bioavailabilities of protein and peptide drugs. Recent developments have been summarised in a number of reviews (Humphrey and Ringrose, 1986; Goosen, 1987; Banga and Chien, 1988; Eppstein and Logenecker, 1988; Lee, 1988; Zhou and Li Wan Po, 1990a).

In an earlier study from this laboratory (Zhou and Li Wan Po, 1990b), it was shown that the enzymic activities of tissues from different portals of absorption, in the rat, differed significantly from each other indicating that one route of absorption may be better than others because of

Correspondence: A. Li Wan Po, Drug Delivery Research Group, School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Rd, Belfast BT9 7BL, U.K.

lower esterase or peptidase activity. The implication is that the choice of a particular route of delivery for a given drug has to be tailored to take account of the enzyme-labile groups present as well as of the inherent permeabilities of the tissues to the drug. For peptide or protein drugs, this information is important since a range of approaches is necessary for improving their bioavailabilities.

If information about the relative metabolic activities of tissues from different absorption sites derived from animal models is to be used for predicting absorption efficiency of those sites in humans, it is important that there is a good correlation between the values obtained in animal and man. This requirement is separate from that necessary for scaling from animal to man (Boxenbaum and D'Souza, 1990). Therefore, as an extension to our previous work, we investigated species differences in metabolic activities of different tissues from different animals. Although ideally human tissues should be used to define absorption barriers, this is not pratical, in vitro, as viable tissues are required and biopsy tissues are not available to us in sufficient quantities for the necessary work. In vivo work is possible but is expensive because of the controls required to account for cross-over effects and inter-subject variability. It would however be necessary for final validation. In this report, work focussing on a comparison of the enzymic activity of tissues from different animal species is described in the hope that the information may help in the selection of the most appropriate model for human tissues.

Materials and Methods

L-Leucine-β-naphthylamide dihydrochloride, *p*-dimethylaminobenzaldehyde (PDAB), (β-naphthylamide dihydrochloride, *p*-nitrophenyl acetate and *p*-nitrophenol were obtained from Sigma (Poole, U.K.). HPLC methanol used in this work was purchased from FSA Laboratory supplies (Loughborough, U.K.). Folin & Ciocalteu's phenol reagent was obtained from BDH (Poole, U.K.). Bovine albumin (fraction *V* powder, 96–99% albumin) was purchased from Sigma. The other reagents and chemicals were analytically pure grade.

Apparatus

Absorbance measurements were carried out with a recording spectrophotometer (PU 8720 UV/Vis scanning spectrophotometer with Video-RGB Monitor CM 8533) equipped with a printer/plotter (Philips, Model C).

Preparation of tissue homogenates

The method used was the same as described previously (Zhou and Li Wan Po, 1990b) except that the rat tissues were supplemented by guinea-pig, rabbit and dog tissues. Except for the rat and guinea-pig tissues, the other tissues were donated by colleagues from other laboratories after killing their animals at the end of their own experiments. This was resorted to in order to minimise use of laboratory animals. The rats were Wistar rats weighing 350–415 g. The guinea-pigs were Duncan Hartley weighing 700–900 g. The rabbits were New Zealand white rabbits weighing 1.5–2 kg and the dogs were greyhounds weighing 15–20 kg. All the animals were allowed free access to water but were deprived of food for 24 h prior to the study. Excised tissues from the freshly killed animals were rinsed with distilled water, blotted dry with absorbent tissues, and homogenised. The 10% w/v tissue homogenates were centrifuged at $7500 \times g$ at 4°C for 15 min and the supernatant stored at −18°C until required for analysis which was always within 1 week. During this period of storage, except for dog tissue esterase, no significant decrease in enzymic activity was observed.

Determination of leucine aminopeptidase

The method used was the same as described in our previous work (Zhou and Li Wan Po, 1990b) based on that reported by Goldberg and Rutenberg (1958) and Takenaka and Takahashi (1962).

Determination of cholesterol esterase activity

Cholesterol esterase activity was determined by the method described by Sih et al. (1963) and modified as decribed in our previous report (Zhou and Li Wan Po, 1990b).

Determination of protein content

Protein concentration in the supernatant solution was measured by the method proposed by Lowry et al. (1951) using bovine serum albumin as reference compound.

Statistical analysis

One-way analyses of variance (ANOVA) were used for testing the null hypothesis of no difference in cholesterol esterase activity, aminopeptidase activity or protein concentration in the tissue homogenates.

When ANOVA led to rejection of the null hypothesis, multiple-range testing was carried out using the Neuman-Keuls Method (Elliott, 1986).

Results and Discussion

Measurement of the esterase activity of the tissues involved hydrolysis of *p*-nitrophenyl acetate and monitoring the absorbance of the solution. To check for linearity in response the plots shown in Fig. 1 were produced. Similar plots (Fig. 2) were constructed for measuring aminopeptidase activity using the method reported by Goldberg and Rutenberg (1958) and modified by Takenaka and Takahashi (1962). It can be seen that for each tissue there was a good linear response between dose and absorbance for each enzyme.

Using the method described, the esterase activities of the different tissues from the rat, rabbit and guinea-pig are shown in Fig. 3. Results for the

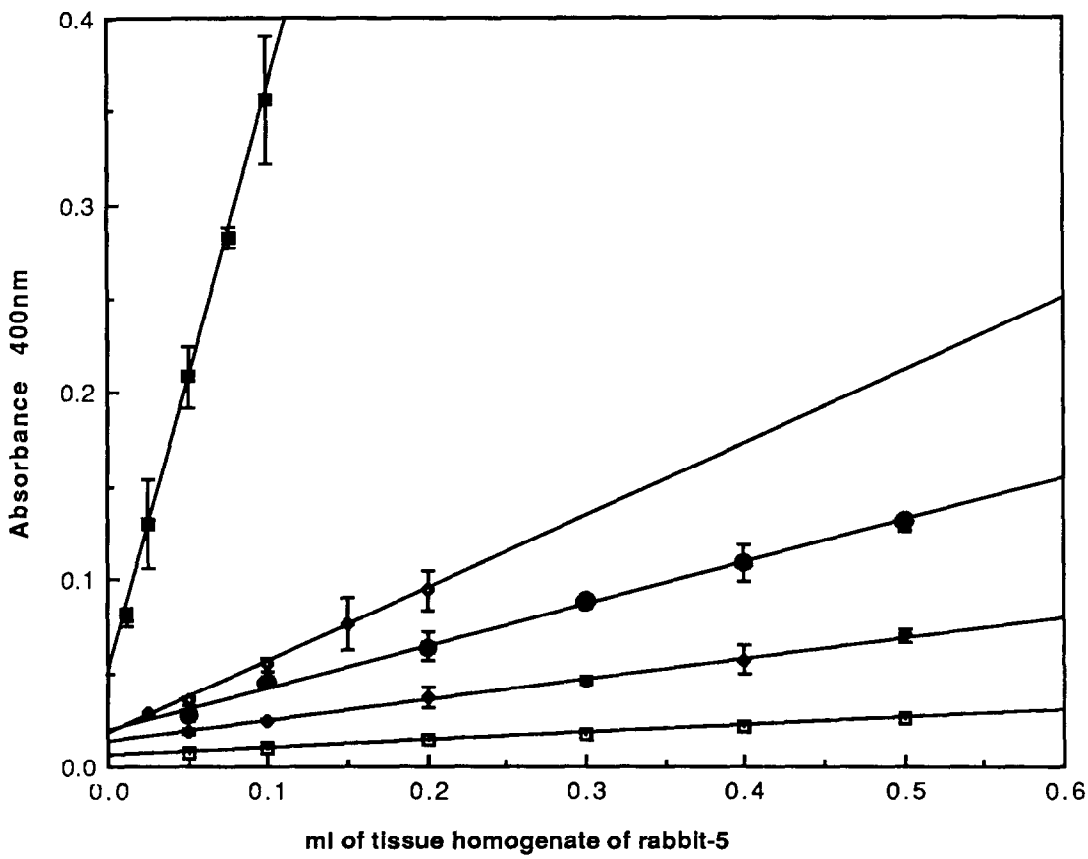

Fig. 1. Typical dose-response plots for the determination of esterase activity. (□) Dermal, (◆) nasal, (●) buccal, (◆) rectal, (■) intestinal.

aminopeptidase activity of the different tissues from the rat, rabbit, guinea-pig and the dog are shown in Fig. 4. Results of measurements of the protein contents of the supernatants of tissue homogenates from the four different animals are shown in Fig. 5.

Analysis of variance (ANOVA) to evaluate the statistical significance of the results obtained were carried out and the resulting ANOVA tables are shown in Table 1. Separate tables are given for each animal species (A–C) and for each tissue studied. When a statistical significance is identified, a multiple range test (Neuman-Keuls) was subsequently carried out for paired comparison. The results are shown in Table 2.

To evaluate whether there was any difference in the enzymic activities of tissues from different animals, the appropriate analyses of variance were carried out to be followed by the corresponding multiple range test when a statistically significant effect was observed in the former test. The results of the paired comparisons are shown in Table 3.

In all the measurements of the enzymic activities of the tissues concerned, the values measured were within the linear dose-response range shown by the calibration curves in Figs 1 and 2.

The relative esterase activities of the different tissues show a different trend in the rat when compared with corresponding data obtained in studies with the rabbit and guinea-pig (Fig. 3). In the rat, dermal tissues appear to possess a higher esterase activity than do nasal, buccal and rectal tissues respectively. In the rabbit and the guinea-pig, the reverse order to that seen in the rat was

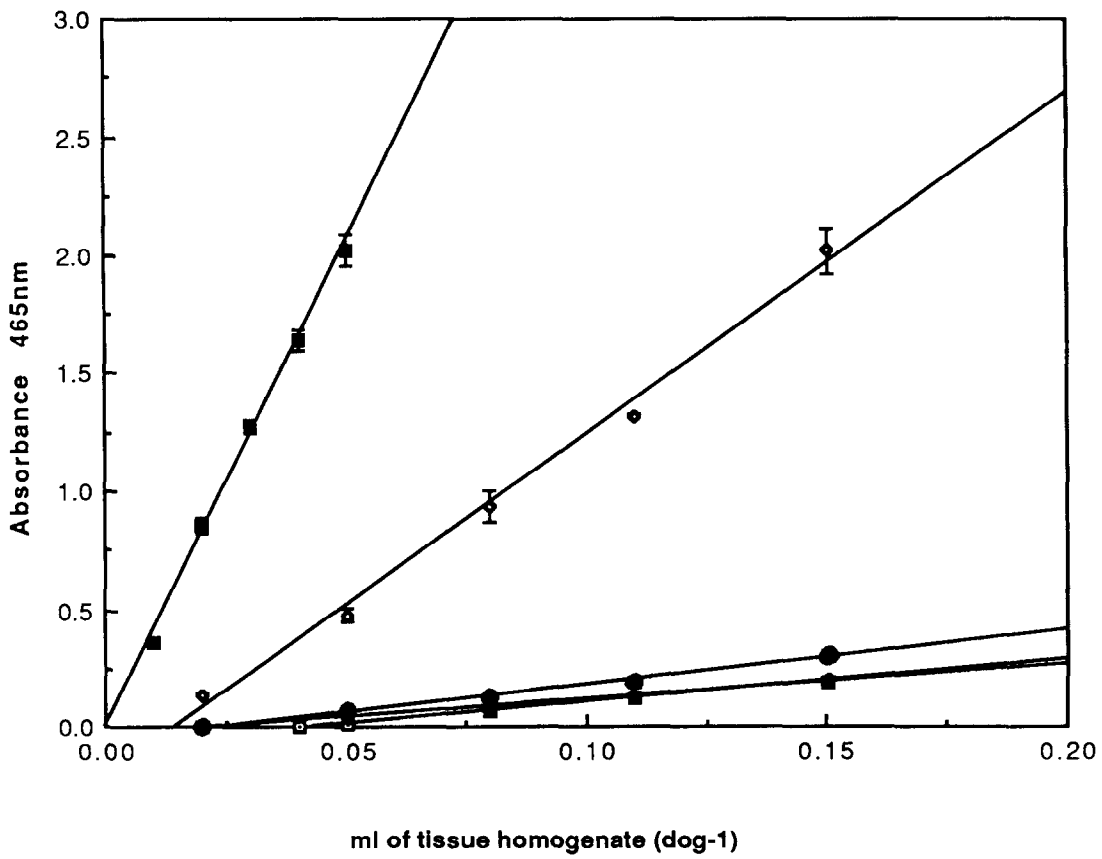

Fig. 2. Typical response plots for measuring aminopeptidase activity of tissue homogenates. (⊡) Dermal, (◆) nasal, (●) buccal, (◆) rectal, (■) intestinal.

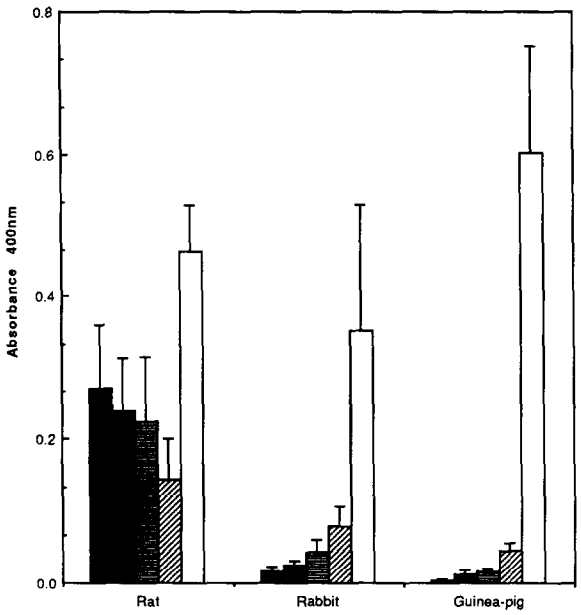

Fig. 3. A comparison of crude esterase activities (mean ± S.D.) of different tissue homogenates from different animals ($n = 5$) (■) Dermal, (▨) nasal, (▤) buccal, (▨) rectal, (□) intestinal.

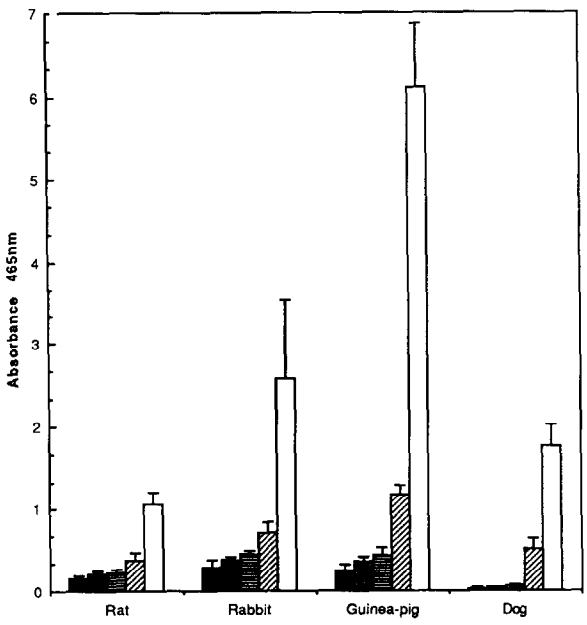

Fig. 4. A comparison of crude aminopeptidase activities (mean ± S.D., $n = 5$) of different tissues homogenates from different animals. (■) Dermal, (▨) nasal, (▤) buccal, (▨) rectal, (□) intestinal.

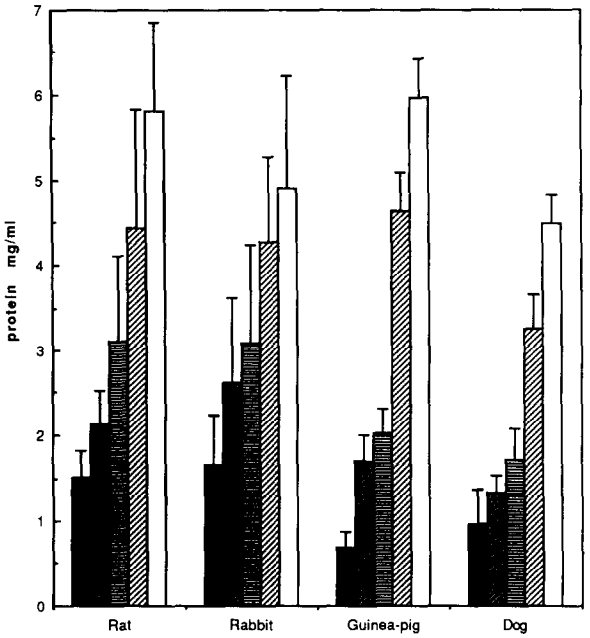

Fig. 5. Comparative protein concentration (mean ± S.D.) from 10% w/v tissue homogenates from different animals. (■) Dermal, (▨) nasal, (▤) buccal, (▨) rectal, (□) intestinal.

observed. Intestinal tissues, however, consistently showed the highest esterase activity. The esterase activity of dog tissues showed poor stability on storage and consistent results were not obtained from day to day. Those results were therefore discarded.

Statistical evaluation of the results (Table 1) showed that despite the differences in rank ordering in the rabbit and guinea-pig, except for intestinal tissues, none of the other tissues showed any statistical difference in esterase activity both with and without adjustment for protein concentration.

With aminopeptidase, the crude activity of the rabbit tissues mirrored those seen with the esterase activity. Only in the intestinal tissue was aminopeptidase significantly higher than the other tissues.

In the dog, dermal, nasal and buccal tissues showed no statistical difference in aminopeptidase activity either before or after adjustment for protein content. They were, however, less active in this respect than rectal tissue which in turn was less active than intestinal tissue. The same result was observed in the guinea-pig. It would therefore

TABLE 1

Analysis of variance tables for comparison of animal buccal, dermal, nasal, rectal and intestinal tissues

Animal	Analysis of variance table				
Source	df	SS	MS	F	Approx. *P*
(A) Rabbit					
(i) Response variable: soluble protein concentration					
Treatment (tissue type)	4	4.04	1.01	23.21	< 0.001
Residual	70	3.04	0.04		
Total	74	7.08			
(ii) Response variable: crude aminopeptidase activity					
Treatment (tissue type)	4	55.91	13.98	76.43	< 0.001
Residual	70	12.80	0.18		
Total	74	68.71			
(iii) Response variable: crude cholesterol esterase activity					
Treatment (tissue type)	4	1.21	0.30	47.62	< 0.001
Residual	70	0.44	0.01		
Total	74	1.65			
(iv) Response variable: specific aminopeptidase activity					
Treatment (tissue type)	4	39.48	9.87	36.90	< 0.001
Residual	70	18.72	0.27		
Total	74	58.20			
(v) Response variable: specific cholesterol esterase activity					
Treatment (tissue type)	4	0.95	0.24	53.97	< 0.001
Residual	70	0.31	0.00		
Total	74	1.26			
(B) Dog					
(i) Response variable: soluble protein concentration					
Treatment (tissue type)	4	5.24	1.31	269.12	< 0.001
Residual	70	0.34	0.00		
Total	74	5.58			
(ii) Response variable: crude aminopeptidase activity					
Treatment (tissue type)	4	33.11	8.28	462.69	< 0.001
Residual	70	1.25	0.02		
Total	74	34.36			
(iii) Response variable: specific aminopeptidase activity					
Treatment (tissue type)	4	37.25	9.31	332.51	< 0.001
Residual	70	1.96	0.03		
Total	74	39.21			
(C) Guinea-pig					
(i) Response variable: soluble protein concentration					
Treatment (tissue type)	4	11.69	2.92	560.78	< 0.001
Residual	70	0.36	0.01		
Total	74	12.05			
(ii) Response variable: crude aminopeptidase activity					
Treatment (tissue type)	4	378.23	94.66	770.79	< 0.001
Residual	70	8.60	0.12		
Total	74	387.20			

(continued)

TABLE 1 (continued)

Animal	Analysis of variance table				
Source	df	SS	MS	F	Approx. P
(C) Guinea-pig					
(iii) Response variable: crude cholesterol esterase activity					
Treatment (tissue type)	4	4.08	1.02	227.92	< 0.001
Residual	70	0.31	0.00		
Total	74	4.40			
(iv) Response variable: specific aminopeptidase activity					
Treatment (tissue type)	4	181.58	45.39	173.38	< 0.001
Residual	70	18.33	0.26		
Total	74	199.91			
(v) Response variable: specific cholesterol esterase activity					
Treatment (tissue type)	4	2.56	0.64	278.00	< 0.001
Residual	70	0.16	0.00		
Total	74	2.72			

appear that as far as aminopeptidase activity was concerned, species differences were more modest than with esterase activity. Indeed, dermal tissues were the least active in all species studied and intestinal, the most active. Rectal tissues were also consistently the second most active source of peptidase activity. Nasal and buccal tissues showed some change in rank order of aminopeptidase activity but this was probably a reflection of the small differences in their relative activities. Indeed, in none of the species studied did the difference reach statistical significance.

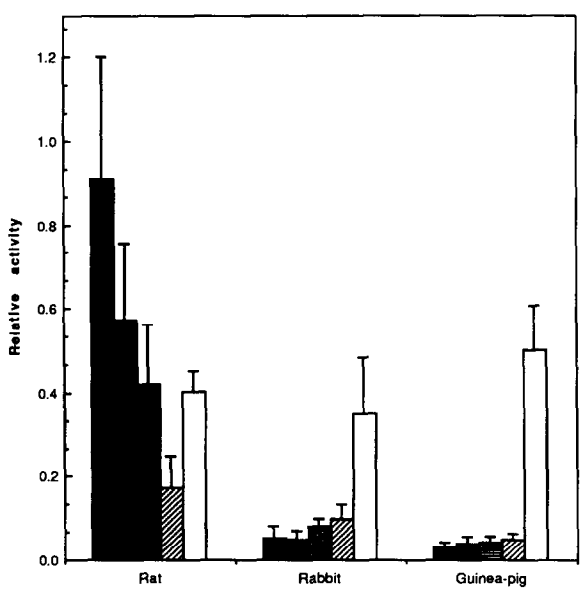

Fig. 6. Comparative esterase activity (mean ± S.D., $n = 5$) of different tissues from different animals corrected for protein concentration. (■) Dermal, (▨) nasal, (▤) buccal, (▨) rectal, (□) intestinal.

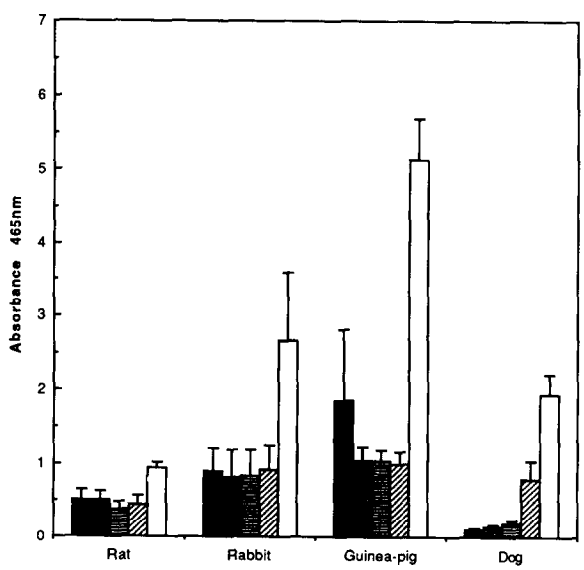

Fig. 7. A comparison of aminopeptidase activity (mean ± S.D., $n = 5$) of different tissues from different animals corrected for protein concentration. (■) Dermal, (▨) nasal, (▤) buccal, (▨) rectal, (□) intestinal.

TABLE 2

Results of multiple range tests (Newman-Keuls) in comparative studies of animal buccal, dermal, nasal, rectal and intestinal tissues

Animal	Tissue				
	Dermal	Nasal	Buccal	Rectal	Intestinal
(A) Rabbit					
(i) Response variable: protein concentration					
Population 1				———	———
Population 2		———	———		
Population 3	———				
(ii) Response variable: crude cholesterol esterase activity					
Population 1	----------	----------	----------	----------	
Population 2					---------
(iii) Response variable: specific cholesterol esterase activity					
Population 1	----------	----------	----------	----------	
Population 2					---------
(iv) Response variable: crude aminopeptidase					
Population 1		----------	----------	----------	
Population 2	----------	----------	----------		
Population 3					---------
(v) Response variable: specific aminopeptidase					
Population 1	----------	----------	----------	----------	
Population 2					---------
(B) Dog					
(i) Response variable: protein concentration					
Population 1	------				
Population 2		------			
Population 3			------		
Population 4				------	
Population 5					---------
(ii) Response variable: crude aminopeptidase					
Population 1	----------	----------	----------		
Population 2				-------	
Population 3					---------
(iii) Response variable: specific aminopeptidase					
Population 1	----------	----------	----------		
Population 2				-------	
Population 3					---------
(C) Guinea-pig					
(i) Response variable: protein concentration					
Population 1	------				
Population 2		------			
Population 3			------		
Population 4				-------	
Population 5					---------
(ii) Response variable: crude aminopeptidase					
Population 1	----------	----------	----------		
Population 2				-------	
Population 3					---------

(continued)

TABLE 2 (continued)

Animal	Tissue				
	Dermal	Nasal	Buccal	Rectal	Intestinal
(C) Guinea-pig					
(iii) Response variable: crude cholesterol esterase activity					
Population 1	----------				
Population 2					--------
(iv) Response variable: specific aminopeptidase					
Population 1	----------				
Population 2				-------	
Population 3					--------
(v) Response variable: specific cholesterol esterase activity					
Population 1	----------				
Population 2					--------

Groups underlined with the same continuous line show no statistical difference at the 5% significance probability level.

The differences in rank ordering of the different tissues, based on their enzymic activities, would suggest that species differences are to be expected when comparing the same tissues across the different species. This is in fact seen to be the case in Figs 3–7. Statistical analyses (Table 3) show that dermal, nasal and buccal tissues show the same species ranking with respect to enzymic activity. Thus, for both crude and protein-normalised aminopeptidase activities, the species ranking was dog < rat < guinea-pig < rabbit. With respect to esterase activity the relative ranking was guinea-pig < rat < rabbit.

Rectal and intestinal tissues showed a different ranking order to that seen with the other tissues studied, with the order for aminopeptidase activity being rat < dog < rabbit < guinea-pig. With intestinal tissue, the rank order for esterase activity was guinea-pig < rat < rabbit. Indeed adjustment for protein content produced yet another ordering for intestinal esterase activity. This time the relative activities were rabbit < rat < guinea-pig.

The differences in enzymic activities between the same tissues in different animals are not surprising. Interspecies pharmacokinetic scaling is, for example, well known (Boxenbaum and D'Souza, 1990). However the differences in species rank ordering with different tissues is less well documented.

What are the implications of the results reported in the present study? The data would suggest that as far as peptidase activity is concerned there is good species to species rank ordering in relative activity of the different tissues studied. Dermal tissues are generally the least active and intestinal tissues the most active. Rectal tissues are the second most active in this respect. The present work was initiated as part of a programme intended to identify optimum routes for drug delivery. For delivering pharmacologically active peptides, the present results suggest that inhibition of peptidase activity, with agents such as aprotinin, would be important if the gastrointestinal route is used. Peptide transport across the epidermal barrier is difficult due to the resistant diffusional barrier presented by the stratum corneum. However, the current work would suggest that should resistance to that barrier be overcome, such as by iontophoresis (Rolf, 1987; Siddiqui et al., 1987; Liu et al., 1988; Srinivasan et al., 1989), then peptidase challenge is likely to be less pronounced than with other tissues.

With esterase activity the species comparisons yielded more complex result in that the rank ordering of the tissues showed species differences. However, intestinal tissues were the most active in all the species studied. Therefore, when delivering drugs with hydrolabile ester functions, it would be important to use esterase inhibitors or to shield the drugs using appropriate techniques, such as stereochemical shielding. Such techniques have shown their usefulness in corticosteroid ester de-

TABLE 3

Results of multiple range tests (Newman-Keuls) in comparative studies on animal species incuding rat, rabbit, guinea-pig and dog

(A) Tissue: Dermal

(i) Response variable: protein concentration

	Guinea-pig	Dog	Rat	Rabbit
Population 1				
Population 2				

(ii) Response variable: crude aminopeptidase

	Dog	Rat	Guinea-pig	Rabbit
Population 1				
Population 2				
Population 3				

(iii) Response variable: crude cholesterol esterase activity

	Guinea-pig	Rabbit	Rat
Population 1			
Population 2			

(iv) Response variable: specific aminopeptidase

	Dog	Rat	Rabbit	Guinea-pig
Population 1				
Population 2				
Population 3				
Population 4				

(v) Response variable: specific cholesterol esterase activity

	Guinea-pig	Rabbit	Rat
Population 1			
Population 2			

(B) Tissue: Nasal

(i) Response variable: protein concentration

	Dog	Guinea-pig	Rat	Rabbit
Population 1				
Population 2				
Population 3				

(ii) Response variable: crude aminopeptidase

	Dog	Rat	Guinea-pig	Rabbit
Population 1				
Population 2				
Population 3				

(iii) Response variable: crude cholesterol esterase activity

	Guinea-pig	Rabbit	Rat
Population 1			
Population 2			

(iv) Response variable: specific aminopeptidase

	Dog	Rat	Rabbit	Guinea-pig
Population 1				
Population 2				
Population 3				
Population 4				

(continued)

TABLE 3 (continued)

(B) Tissue: Nasal

(v) Response variable: specific cholesterol esterase activity

	Guinea-pig	Rabbit	Rat
Population 1	----------		
Population 2			---------

(C) Tissue: Buccal

(i) Response variable: protein concentration

	Dog	Guinea-pig	Rabbit	Rat
Population 1			----------	
Population 2	----------			

(ii) Response variable: crude aminopeptidase

	Dog	Rat	Guinea-pig	Rabbit
Population 1			----------	
Population 2	------			
Population 3		------		

(iii) Response variable: crude cholesterol esterase activity

	Guinea-pig	Rabbit	Rat
Population 1	----------		
Population 2			------

(iv) Response variable: specific aminopeptidase

	Dog	Rat	Rabbit	Guinea-pig
Population 1	------			
Population 2		------		
Population 3			------	
Population 4				---------

(v) Response variable: specific cholesterol esterase activity

	Guinea-pig	Rabbit	Rat
Population 1	----------		
Population 2			------

(D) Tissue: Rectal

(i) Response variable: protein concentration

	Dog	Rabbit	Rat	Guinea-pig
Population 1		----------		
Population 2	------			

(ii) Response variable: crude aminopeptidase

	Rat	Dog	Rabbit	Guinea-pig
Population 1	------			
Population 2		------		
Population 3			------	
Population 4				---------

(iii) Response variable: crude cholesterol esterase activity

	Guinea-pig	Rabbit	Rat
Population 1	---------		
Population 2		------	
Population 3			------

(continued)

TABLE 3 (continued)

(D) Tissue: Rectal

(iv) Response variable: specific aminopeptidase

	Rat	Dog	Rabbit	Guinea-pig
Population 1		----------		
Population 2	------			
Population 3				---------

(v) Response variable: specific cholesterol esterase activity

	Guinea-pig	Rabbit	Rat
Population 1	---------		
Population 2		------	
Population 3			----------

(E) Tissue: Intestinal

(i) Response variable: protein concentration

	Guinea-pig	Dog	Rat	Rabbit
Population 1			----------	
Population 2	----------			

(ii) Response variable: crude aminopeptidase

	Rat	Dog	Rabbit	Guinea-pig
Population 1	------			
Population 2		-------		
Population 3			----------	
Population 4				---------

(iii) Response variable: crude cholesterol esterase activity

	Guinea-pig	Rat	Rabbit
Population 1	---------		
Population 2		-------	
Population 3			----------

(iv) Response variable: specific aminopeptidase

	Rat	Dog	Rabbit	Guinea-pig
Population 1	------			
Population 2		-------		
Population 3			----------	
Population 4				---------

(v) Response variable: specific cholesterol esterase activity

	Rabbit	Rat	Guinea-pig
Population 1	----------		
Population 2			---------

sign and betamethasone-17-valerate, for example, is much more active than the corresponding 21-ester (Cheung et al., 1985).

References

Anik, S.T., LHRH preparations for intranasal administration. *Eur. Patent.* Appl. EP. 0 111 841, 83112469 0 (1984).

Banga, A.K. and Chien, Y.W.C., Systemic delivery of therapeutic peptides and proteins. *Int. J. Pharm.*, 48 (1988) 15–50.

Boxenbaum, R. and D'Souza, R.W., Interspecies pharmacokinetic scaling, biological design and neoteny. In Testa, B. (Ed.), *Advances in Drug Research*, Vol. 19, Academic Press, New York, 1990, pp. 139–191.

Cheung, Y.W., Li Wan Po, A. and Irwin, W.J., Cutaneous biotransformation as a parameter in the modulation of the activity of topical corticosteroids. *Int. J. Pharm.*, 26 (1985) 175–189.

Elliott, A.C., Kwikstat, Mission Technol. (PA, U.S.A.) (1986) version 10.

Eppstein, D.A. and Logenecker, J.P., Alternative delivery systems for peptides and proteins as drugs. *CRC. Crit. Rev. Ther. Drug Carr. Syst.*, 5 (1988) 99–139.

Goldberg, J.A. and Rutenberg, A.M. The colorimetric determination of leucine aminopeptidase in urine and serum disease. *Cancer*, 11 (1958) 283–290.

Goosen, M.F.A., Insulin delivery systems and the encapsulation of cell for medical and industrial use. *CRC. Crit. Rev. Biocompat.*, 3 (1987) 1–24.

Humphrey, M.J. and Ringrose, P.S., Peptides and related drugs: A review of their absorption, metabolism, and excretion. *Drug Metab. Rev.*, 17 (1986) 283–310.

Lee, V.H.L., Enzymatic barriers to peptide and protein absorption. *CRC. Crit. Rev. Ther. Drug Carr. Syst.*, 5 (1988) 69–97.

Liu, J.C., Sun, Y., Siddiqui, O. and Chien, Y.W., Blood glucose control in diabetic rats by transdermal iontophoretic delivery of insulin. *Int. J. Pharm.*, 44 (1988) 197–204.

Lowry, O.H., Rosebrough, N.J. and Randall, R.J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193 (1951) 265–275.

Rolf, D., Iontophoresis and promoted drug absorption. *Pharm. Technol.*, 11 (1987) 47–50.

Sandow, J. and Petri, W., Intranasal administration of peptides, biological activity and therapeutic efficacy. In Chien, Y.M. (ed.), *Transnasal Systemic Medications*, Elsevier, Amsterdam, 1985, pp. 183–199.

Siddiqui, O., Sun, Y., Ziu, J.C. and Chien, Y.W., Facilitated transdermal transport of insulin. *J. Pharm. Sci.*, 76 (1987) 341–345.

Sih, C.J., Laval, J. and Rahim, M.A., Purification of properties of steroid esterase from Nocardia restrictus. *J. Biol. Chem.*, 238 (1963) 566–571.

Srinivasan, V., Higuchi, W.I., Sims, S.M., Ghanem, A.H. and Behl, C.R., Transdermal iontophoretic drug delivery: Mechanistic analysis and application to polypeptide delivery. *J. Pharm. Sci.*, 78 (1989) 370–375.

Takenaka, M. and Takahashi, H.A., A new method for the determination of serum leucine aminopeptidase. *Med. Biol.*, 65 (1962) 74–77.

Wood, A.J., Maurer, G., Niederberger, W. and Beveridge, T., Cyclosporine: pharmacokinetics, metabolism and drug interactions. *Transplant. Proc.*, 15 (1983) 2409–2417.

Zhou, X.H. and Li Wan Po, A., Delivery of peptide and protein drugs. *Int. J. Pharm.*, (1990a) submitted.

Zhou, X.H. and Li Wan Po, A., Comparison of enzymic activities of tissues lining portals of drug absorption, using the rat as a model. *Int. J. Pharm.*, 62 (1990b) 259–267.