Table 1. The inhibitory effects of NaNed and SCG on α-MSH and TPA-potentiated α-MSH potency

	NaNed				SCG			
	% inhibition of α-MSH potency ± SEM	N	% inhibition of TPA-potentiated α-MSH potency ± SEM	N	% inhibition of α-MSH potency ± SEM	N	% inhibition of TPA-potentiated α-MSH potency ± SEM	N
5 μM	6.2 ± 5.0	4	57.6 ± 4.0	3	12.3 ± 10.2	6	48.0 ± 8.0	3
50 μM	21.0 ± 9.3	4	66.4 ± 3.7	3	21.5 ± 14.6	7	81.0 ± 4.0	3
500 μM	32.2 ± 7.0	6	84.5 ± 3.2	3	50.6 ± 8.0	7	74.5 ± 6.5	3
5 mM	64.5 ± 6.8	7	89.9 ± 1.4	3	39.4 ± 11.9	6	79.5 ± 5.0	3

derivative, the mechanism which we have found is likely to be a general property of cromolyns which we presume underlies their therapeutic effect. Both the principle and the assay therefore provide a new method for development of more potent mast cell "stabilising" agents.

Acknowledgements—This work was supported by a grant from the MRC. We thank Fisons Pharmaceuticals (UK) for the cromolyns.

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REFERENCES

1. R. E. C. Altouman, *Clin. Allergy* **10**, 481 (1980).
2. E. Wells and J. Mann, *Biochem. Pharmac.* **32**, 837 (1983).
3. R. Sagi-Eisenberg, *TIPS* **6**(5), 198 (1985).
4. R. J. Carter and S. Shuster, *J. Pharm. Pharmac.* **30**, 233 (1975).
5. A. M. McCormack, R. J. Carter, A. J. Thody and S. Shuster, *Peptides* **3**, 13 (1982).
6. R. J. Carter and S. Shuster, *J. invest. Dermatol.* **71**, 229 (1978).
7. W. J. Atwell, *Science N.Y.* **49**, 48 (1919).
8. J. S. Huxley and L. T. Hogben, *Proc. R. Soc. Lond. Ser. B.* **93**, 36 (1922).
9. M. W. Bitensky and S. R. Burstein, *Nature, Lond.* **208**, 1282 (1965).
10. K. Abe, R. W. Butcher, W. E. Nicholson, C. E. Baird, R. H. Liddle and G. W. Liddle, *Endocrinology* **84**, 362 (1969).
11. F. C. G. Van de Veerdonk and T. M. Konijn, *Acta Endocrinology* **64**, 364 (1970).
12. R. J. Carter and S. Shuster, *Clin. Sci.* **60**, 27 (1981).
13. R. J. Carter and S. Shuster, *Br. J. Pharmac.* **75**, 169 (1982).
14. M. Rodbell, *Nature, Lond.* **284**, 17 (1980).
15. M. Castagna, Y. Takai, K. Kaibuchi, K. Sano, U. Kikkaura and Y. Nishizuka, *J. biol. chem.* **257**, 7847 (1982).
16. A. M. Lucas, S. Shuster and A. J. Thody, *Br. J. Derm.* **113**, 770 (1985).
17. K. B. Seamon and J. W. Daly, *J. Cyclic Nucleotide Res.* **7**, 201 (1981).
18. C. J. Bliss, in *Vitamin Methods*, Vol. 2 (Ed. P. Gyorgy), p. 445. Academic Press, New York (1952).
19. M. E. Hadley, B. Anderson, C. B. Heward, T. K. Sawyer and V. J. Hruby, *Science* **213**, 1025 (1981).
20. A. M. Lucas, S. Shuster and A. J. Thody, *J. invest. Derm.* **80**, p367 (1983).
21. A. M. Lucas, A. J. Thody and S. Shuster, *Br. J. Derm.* **109**, p692 (1983).
22. T. C. Theoharides, W. Sieghart, P. Greengard and W. W. Douglas, *Science* **207**, p80 (1980).

Biochemical Pharmacology, Vol. 36, No. 4, pp. 565–567, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

β-Lactam antibiotics and transport via the dipeptide carrier system across the intestinal brush-border membrane

(*Received* 24 *March* 1986; *accepted* 28 *July* 1986)

It is well established that the small intestine can transport amino-β-lactam antibiotics with an α-amino group in the side chain such as amoxicillin, cyclacillin, cephalexin, cephradine and cefadroxil, which have very low lipid solubility, via the dipeptide carrier system(s) [1–5].

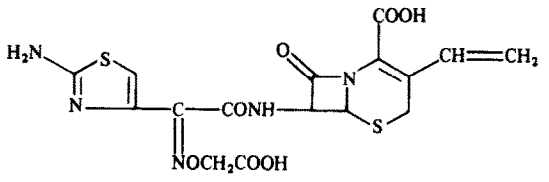
Until now, α-amino-group-deficient β-lactam antibiotics such as benzylpenicillin and propicillin have been believed to be absorbed by simple diffusion restricted by the brush-border membrane lipid barrier [6–8]. However, it is unclear whether an α-amino group on the side chain of the antibiotic is essential for transport by the dipeptide carrier system(s) or not.

The purpose of this study was to characterize the intestinal transport of cefixime* (FK027) (Fig. 1), a new oral cephalosporin antibiotic, using the intestinal brush-border membrane vesicles (BBMVs), and to elucidate what kind of β-lactam antibiotics have the potential ability to be transported across the brush-border membrane via the dipeptide carrier system(s).

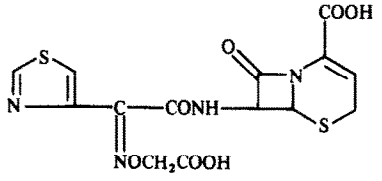
Materials and methods

The β-lactam antibiotics used in this work were supplied as follows: cefixime (CFIX), FK089, ceftizoxime and cefazolin by the Fujisawa Pharmaceutical Co., Osaka, Japan; cyclacillin (ACPC) and propicillin (PPPC) by Takeda Chemical Industries, Osaka; benzylpenicillin (PCG), phenoxymethylpenicillin (PCV) and dicloxacillin (MDIPC) by Meiji Seika Kaisha, Tokyo, Japan; cephradine by the Sankyo Co., Tokyo; D-cephalexin (D-CEX) by Shionogi & Co., Osaka; carbenicillin by the Taito Pfizer Co., Tokyo; and L-cephalexin (L-CEX, custom-made) by the Asahi Chemical Industry Co., Tokyo.

* Abbreviations: cefixime, (6*R*,7*R*)-7-[(Z)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)-acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylicacid; FK089, (6*R*,7*R*)-7-[(Z)-2-(4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylic acid; and HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.



Cefixime



FK089

Fig. 1. Structures of cefixime (CFIX) and FK089.

All other chemicals were of reagent grade and were used without further purification.

Intestinal BBMVs were prepared following the method of Kessler *et al.* [9]. Uptake of CFIX into BBMVs was measured by the rapid filtration method as reported by Hopfer *et al.* [10]. The conditions for the uptake experiments are described in the figure legends and table footnote.

CFIX trapped on a Millipore filter was extracted with 500 μl of distilled water and analyzed by high-performance liquid chromatography (HPLC). The HPLC system was equipped with a constant flow pump, BIP-I and UV detector, UVIDEC 100-III (Japan Spectroscopic Co., Tokyo, Japan). The analytical column used was Cosmosil 5-C_{18} (4.6 mm × 15 cm, Nakarai Chemicals Ltd., Kyoto, Japan). The mobile phase, a mixture of acetonitrile and water (2.75/7.25, v/v) containing 0.01 M ammonium acetate and 0.01 M tetra-*N*-butylammonium bromide, was used at a flow rate of 1.0 ml/min and was monitored at 290 nm. Protein was measured by the method of Lowry *et al.* [11] with bovine serum albumin used as a standard.

Results and discussion

Table 1 summarizes the inhibitory effects of peptides, amino acids and β-lactam antibiotics on CFIX uptake into BBMVs in the presence of a proton gradient (extravesicular pH = 5.0; intravesicular pH = 7.5) and in the absence of sodium ion. There was no significant inhibition by any amino acids. It is significant that CFIX uptake was inhibited to various extents by the peptides and β-lactam antibiotics, since the results suggest the existence of a common transport system between CFIX and the peptides or the other β-lactam antibiotics. L-CEX inhibited the uptake of CFIX 2.5 times more than did D-CEX, indicating that the transport system responsible for CFIX is highly stereospecific.

The inhibitory effects of glycyl-L-proline (Gly-Pro), FK089, PCG and ACPC on the transport of CFIX were further examined kinetically (Fig. 2). The peptide and these antibiotics inhibited the uptake of CFIX competitively, and the estimated inhibitor constants, K_i, were 5.29 ± 0.95, 1.90 ± 0.48, 1.51 ± 0.22 and 1.28 ± 0.14 mM, respectively. The observed competitive inhibition kinetics and the intestinal transport characteristics established previously for ACPC [3] and Gly-Pro [12] suggest that CFIX has a common affinity site with these β-lactam antibiotics on the peptide carrier protein.

Table 1. Inhibitory effects of peptides, amino acids and β-lactam antibiotics on the uptake of cefixime (2 mM) into intestinal BBMVs

Inhibitor	Inhibitor concn (mM)	Relative uptake (% of control)
Control		100.0
Glycyl-L-proline	50	16.7 ± 3.7*
Glycylsarcosine	50	58.1 ± 5.0*
Glycine	50	87.9 ± 6.5
Proline	50	96.8 ± 16.5
Glutamic acid	50	110.0 ± 4.4
Lysine	50	110.0 ± 12.1
Benzylpenicillin	20	15.9 ± 1.3*
Carbenicillin	20	46.5 ± 5.5*
Dicloxacillin	20	26.9 ± 5.0*
Phenoxymethylpenicillin	20	30.0 ± 14.5*
Propicillin	20	53.3 ± 16.4*
Cefazolin	20	74.0 ± 7.9*
Ceftizoxime	20	67.1 ± 12.8*
FK089	4	36.7 ± 10.0*
Cyclacillin	20	53.6 ± 12.0*
D-Cephalexin	25	68.4 ± 1.7*
L-Cephalexin	25	24.4 ± 1.4*
Cephradine	20	61.7 ± 1.8*

Membrane vesicles were preloaded with 10 mM Tris–HEPES buffer (pH 7.5) containing 270 mM mannitol. Uptake of 2 mM cefixime was measured at 37° for 30 sec by the addition of the membrane vesicles to 10 mM Tris–citrate buffer (pH 5.0) containing mannitol alone as a control or containing mannitol and inhibitors. Each value, expressed as the percentage of the control, is the mean ± SE of three to five experiments. The control value (100%) for cefixime uptake was 2.85 ± 0.16 nmol/30 sec per mg protein.

* The level of significance, determined by Student's *t*-test, was set at $P < 0.05$.

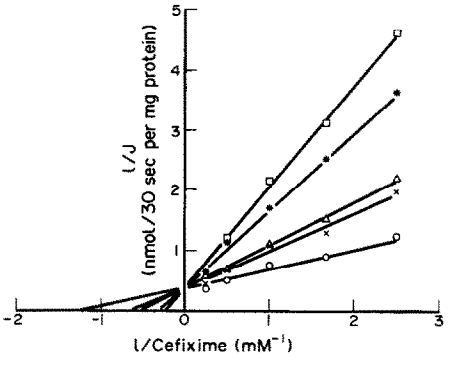


Fig. 2. Lineweaver–Burk plots of CFIX uptake into BBMVs showing inhibition by Gly-Pro and β-lactam antibiotics. A 20-μl sample of membrane vesicles was preloaded with 10 mM Tris–HEPES buffer (pH 7.5) containing 270 mM mannitol (buffer A). Uptake of CFIX (0.4 to 4 mM) was measured at 37° for 30 sec with the addition of a 20-μl sample of the membrane vesicles to 80 μl of 10 mM Tris–citrate buffer (pH 5.0) containing 270 mM mannitol (buffer B) in the presence of 5 mM Gly-Pro (□), 2 mM FK089 (△), 5 mM PCG (●) and 5 mM ACPC (▽) or in the absence of inhibitors (○). Each point represents the mean of three to five experiments.

To confirm the existence of a common carrier system between CFIX and the peptides or the other β-lactam antibiotics in the brush-border membrane, some of the inhibitors were examined for a countertransport effect. The results are shown in Fig. 3, expressed as the ratio, in percent, of uptake after 10 min to that in the control experiment which was performed simultaneously. The initial uptake of CFIX was stimulated by preloading of Gly-Pro and both β-lactam antibiotics with (ACPC) and without (FK089) an α-amino group in the side chain, whereas uptake at the steady state (10 min) was not altered.

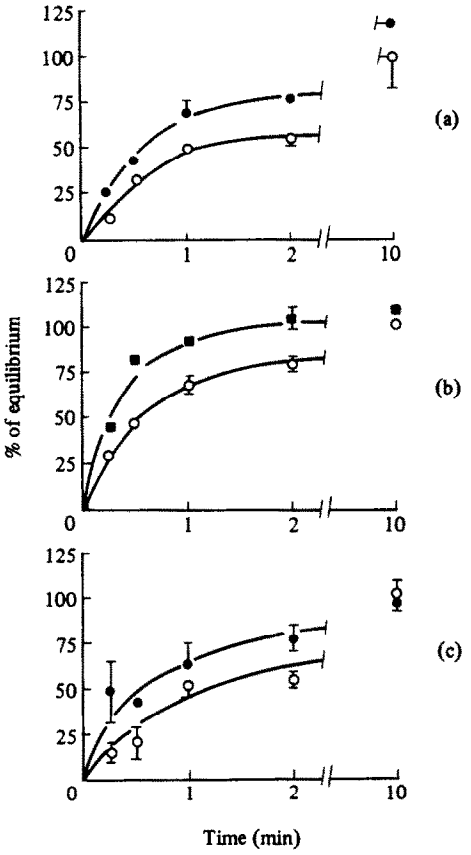


Fig. 3. Countertransport effect of (a) Gly-Pro, (b) FK089 and (c) ACPC on CFIX uptake into BBMVs. A 20-μl sample of membrane vesicles dispersed in 10 mM Tris–citrate buffer (pH 5.0) containing 270 mM mannitol (buffer B) was preloaded at 37° for 10 min with the addition of 20 μl of (a) 8 mM Gly-Pro, (b) 8 mM FK089 or (c) 10 mM ACPC dissolved in buffer B (●) or 20 μl of buffer B alone as a control (○). Uptake of 2 mM CFIX into BBMVs was started by adding 360 μl of a solution of cefixime dissolved in buffer B. The mixtures were incubated at 37° for 10 min. The ordinate is expressed as the ratio, in percent, of uptake after 10 min to that of the control. Each point represents the mean ± SE of three to five experiments. The control value (100%) for CFIX uptake was 2.15 ± 0.56 nmol per mg protein.

The clearly obtained competitive inhibitory effect and the countertransport effect indicate that all types of β-lactam antibiotics share a common transport system with peptides in the intestinal brush-border membrane. Although the chemical structure essential for the translocation of β-lactam antibiotics via the peptide transport system is unclear from the present study, it can be safely said that β-lactam antibiotics themselves have various affinity constants for a peptide carrier system(s) depending on the structural differences in the side chains at the 3- and 7-position of cephems and at the 6-position of penicillins. The translocation of β-lactam antibiotics possessing sufficient lipid solubility such as PCG, PCV, PPPC and MDIPC through the brush-border membrane may occur via either the peptide transport carrier and/or via the lipid membrane barrier.

Acknowledgement—The authors wish to thank Mr. Yuki Kin for his technical assistance.

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REFERENCES

1. J. M. Addison, D. Burston, J. A. Dalrymple, D. M. Matthews, J. W. Payne, M. H. Sleisenger and S. Wilkinson, *Clin. Sci. molec. Med.* **49**, 313 (1975).
2. T. Kimura, H. Endo, M. Yoshikawa, S. Muranishi and H. Sezaki, *J. Pharmacobio-Dynamics* **1**, 262 (1978).
3. E. Nakashima, A. Tsuji, H. Mizuo and T. Yamana, *Biochem. Pharmac.* **33**, 3345 (1984).
4. E. Nakashima, A. Tsuji, S. Kagatani and T. Yamana, *J. Pharmacobio-Dynamics* **7**, 452 (1984).
5. T. Okano, K. Inui, M. Takano and R. Hori, *Biochem. Pharmac.* **35**, 1781 (1986).
6. A. Tsuji, E. Miyamoto, N. Hashimoto and T. Yamana, *J. pharm. Sci.* **67**, 1705 (1978).
7. A. Tsuji, E. Miyamoto, O. Kubo and T. Yamana, *J. pharm. Sci.* **68**, 812 (1979).
8. A. Tsuji and T. Yamana, in *Beta-Lactam Antibiotics* (Ed. S. Mitsuhashi), p. 235. Japan Science Societies Press, Tokyo (1981) (Engl.).
9. M. Kessler, O. Acuto, C. Storelli, H. Murer, M. Muller and G. Semenza, *Biochim. biophys. Acta* **506**, 136 (1978).
10. U. Hopfer, K. Nelson, J. Perrotto and K. J. Isselbacher, *J. biol. Chem.* **248**, 25 (1973).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
12. V. Ganapathy and F. H. Leibach, *J. biol. Chem.* **258**, 14189 (1983).