amines from adrenal medulla (Dadkar et al 1984). These results suggest that pressor effect of cimetidine may counteract the hypotensive effect of clonidine.

In the present study, we have demonstrated that in anaesthetized SH rats, pretreatment with the H_2-receptor antagonist, cimetidine (i.c.v.) inhibited the fall in blood pressure induced by intravenous injection of clonidine. In addition, the inhibition of the hypotensive effect of clonidine by cimetidine is found to be dependent on its pressor effect. From this finding, it seems reasonable to assume that increased peripheral sympathetic activity by cimetidine may be responsible for inhibition of the hypotensive action of clonidine.

Furthermore, it has been demonstrated that central administration of cimetidine causes a sustained rise in perfusion pressure in autoperfused hind quarters of SH rats, and it was suggested that cimetidine might be increasing the vascular resistance due to alteration in the neurogenic tone of hindlimb vasculature (Dohadwalla & Dadkar 1981). To investigate the possibility of involvement of peripheral action of cimetidine in countering the hypotensive effect of clonidine, further interaction studies were performed using different antihypertensive agents which are known to elicit blood pressure lowering effect either by interruption of peripheral sympathetic tone or by direct action on the vascular smooth muscle. It was observed that cimetidine (250 μg i.c.v.) significantly counteracted the established hypotensive action of minoxidil, pentolinium and guanethidine, which are supposed to act mainly at the level of peripheral vasculature (DuCharme et al 1973: Willems & Bogaert 1978).

From these observations, it can be concluded that i.c.v. administration of cimetidine results in peripheral vasoconstriction and this may offer resistance to hypotensive actions not only of clonidine, but also to other hypotensive agents. Moreover, these findings also reject the hypothesis that clonidine initiates its central hypotensive effect via histamine H_2-receptors.

REFERENCES

Dadkar, N. K., Aroskar, V. A., Gupte, R. D., Dohadwalla, A. N. (1984) J. Pharm. Pharmacol. 36: 488–490

Dohadwalla, A. N., Dadkar, N. K. (1981) 8th International Congress of Pharmacology, Tokyo, July 19–24, Abstr. 093: 847

DuCharme, D. W., Freyburger, W. A., Graham, B. E., Carlsen, R. S. (1973) J. Pharmacol. Exp. Ther. 184: 662–670

Finch, L., Harvey, C. A., Hicks, P. E., Owen, D. A. A. (1978) Neuropharmacology 17: 307–313

Karppanen, H., Paakkari, I., Huotari, R., Orma, A. L. (1976) Nature 259: 587–588

Karppanen, H., Paakkari, I., Paakkari, P. (1977) Eur. J. Pharmacol. 42: 299–302

Nomura, Y., Segawa, T. (1979) Br. J. Pharmacol. 66: 531–535

Okamoto, K., Aoki, K. (1963) Jap. Circulation 27: 282–293

Pilc, A., Gulembiowska-Nikitin, K., Vetulani, J. (1979) Eur. J. Pharmacol. 56: 177–178

Timmermans, P. B. M. W. M., Karamat Ali, F., Kossen, S. P., van Zwieten, P. A. (1980) J. Pharm. Pharmacol. 32: 147

Vogt, M. (1977) Br. J. Pharmacol. 61: 441–443

Willems, J. L., Bogaert, M. G. (1978) Gen. Pharmacol. 9: 223–227

J. Pharm. Pharmacol. 1985, 37: 374–375
Communicated October 9, 1984

Uptake of amino β-lactam antibiotics into rat intestinal brush border membrane vesicles

KEN ISEKI, AKIHIRO IEMURA, KATSUMI MIYAZAKI, TAKAICHI ARITA*, *Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo, 060, Japan*

Uptake of amino β-lactam antibiotics into rat intestinal brush border membrane vesicles has been examined for characterization of the transport of the antibiotics through the gut wall of the rat. The uptake of cephradine, cephalexin and ampicillin into membrane vesicles was similar, and there were no significant changes in the uptake in the presence of a NaCl or a KCl gradient. These results suggested that the carrier-mediated transport systems relating to amino acids and glucose were not concerned with the intestinal absorption mechanism of amino β-lactam antibiotics.

Amino β-lactam antibiotics are absorbed from the intestinal lumen after oral administration despite poor lipophilicity. Recent findings suggested that the intestinal absorption of antibiotics such as cephalexin and cephradine involved a carrier-mediated transport system relating to amino acids and dipeptides (Kimura et al 1978, 1982). However, in a preliminary experiment, we found that in the presence of several amino acids and dipeptides there were no changes in the absorption of these antibiotics. We have therefore used membrane vesicles for the investigation and characterization of amino β-lactam antibiotics transport through the brush border membranes of rat small intestine.

* Correspondence.

Method

Brush border membrane vesicles were isolated from the intestine of male Wistar rats (180–230 g) according to the calcium chloride precipitation method of Kessler et al (1978). The membranes were suspended to a final concentration of about 4 mg protein ml^{-1} with 20 mM

HEPES/Tris buffer (pH 7·5) and 100 mM mannitol. The purity of the membranes was routinely evaluated by the enrichment of alkaline phosphatase (E.C. 3.1.3.1), an enzyme specific for the intestinal brush border membrane. The specific activity of this enzyme increased 13-fold in the final membrane suspension over the homogenate of intestinal scrappings. Protein was determined by the method of Lowry et al (1951) with bovine serum albumin as the standard.

The uptake of substrate was measured by a rapid filtration technique. Membrane vesicles were preincubated at 25 °C for 10 min. Incubation media contained, in a 200 μl final volume, 20 mM HEPES/Tris buffer (pH 7·5), 100 mM mannitol, 100 mM NaCl or KCl, and 1 mM substrates. The reaction was initiated by the addition of 100 μl of brush border membrane vesicle suspension. At intervals, the incubation was stopped by diluting a reaction mixture with 8 ml of ice-cold buffer (1 mM Tris/Cl, pH 7·5 and 150 mM NaCl). The diluted samples were immediately filtered through Millipore filters (HA025, 0·45 μm) and washed with 5 ml of ice-cold buffer.

In separate experiments, non-specific adsorption to the Millipore filter was determined by using the incubation medium instead of brush border membrane suspension. This value was subtracted from uptake data. The filter-trapped membrane vesicles were put into the test tube containing 1 ml of redistilled water.

The drugs trapped on the Millipore filters were determined by high-performance liquid chromatography using fluorometric detection according to Miyazaki et al (1983). For ampicillin, the procedure for cephalexin was followed.

Results and discussion

The effect of extravesicular osmolarity on cephradine and cephalexin uptake at steady-state (60 min) was determined using cellobiose as the impermeant solute. The uptake of both antibiotics was inversely proportional to extravesicular osmolarity. Extrapolation to infinite extravesicular osmolarity (zero intravesicular space) was negligible compared with uptake of the antibiotics into brush border membrane vesicles (unpublished data).

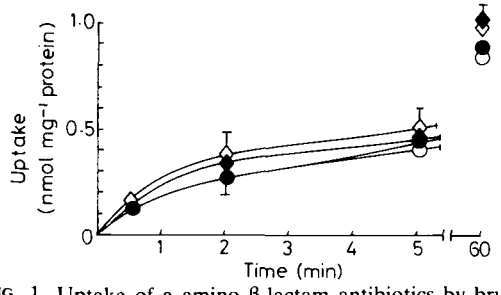

FIG. 1. Uptake of a amino β-lactam antibiotics by brush border membrane vesicles. Membrane vesicles were preincubated at 25 °C in 20 mM HEPES/Tris (pH 7·5) and 100 mM mannitol for 10 min. The vesicles (100 μl) were incubated at 25 °C with the substrate mixture (100 μl) containing 20 mM HEPES/Tris (pH 7·5), 100 mM mannitol, 200 mM NaCl (●◆) or KCl (○◇), and either 2 mM cephalexin (●○), cephradine (◆◇), or ampicillin the plots of which are essentially identical with that for cephalexin. Final concentration: 100 mM NaCl or KCl, 1 mM antibiotics. Each point represents the mean ± s.d. of two experiments performed in triplicate or duplicate determinations, some s.d. values lie within the symbol area.

In order to estimate the general transport characteristics of amino β-lactam antibiotics in the intestinal brush border membranes, cephalexin, cephradine and ampicillin were used for the transport studies. Fig. 1 shows the uptake of these drugs (1 mM) by brush border membrane vesicles in the presence of a 100 mM NaCl or KCl gradient (outside to inside). The rates of uptake for these three antibiotics were almost the same, and there were no significant changes in the uptake into the brush border membrane vesicles in the presence of a NaCl or a KCl gradient.

Inui et al (1983) showed the existence of a carrier-mediated transport system for aminocephalosporins in rat renal brush border membranes. On the contrary, we have found in this study that the uptake rates of cephalexin, cephradine and ampicillin into the intestinal brush border membrane vesicles were the same as those of monobasic cephalosporins which Inui et al found to be simply diffused in renal brush border membranes. It was assumed that the difference in the transport characteristics between the brush border membrane vesicles from the two kinds of tissue were related to the different roles of the tissues. We also found that the membrane vesicles we used maintained the function of a carrier-mediated transport system relating to amino acids and glucose (data was not shown). These results suggested such carrier-mediated transport systems were not concerned with the intestinal absorption mechanism of amino β-lactam antibiotics.

Previously (Miyazaki et al 1982), we reported that these amino β-lactam antibiotics, but not monobasic cephalosporins, had an affinity for one of the soluble fractions from the intestinal mucosa and the correlation of the transport with the binding to this fraction (F1) is being examined.

REFERENCES

Inui, K., Okano, T., Takano, M., Kitazawa, S., Hori, R. (1983) Biochem. Pharmacol. 32: 621–626

Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M., Semenza, G. (1978) Biochim. Biophys. Acta 506: 136–154

Kimura, T., Endo, H., Yoshikawa, M., Muranishi, S., Sezaki, H. (1978) J. Pharm. Dyn. 1: 262–267

Kimura, T., Yamamoto, T., Mizuno, M., Suga, Y., Kitade, S., Sezaki, H. (1983) Ibid. 6: 246–253

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Miyazaki, K., Iseki, K., Arita, T. (1982) J. Pharm. Dyn. 5: 593–602

Miyazaki, K., Ohtani, K., Sunada, K., Arita, T. (1983) J. Chromatogr. 276: 478–482