Biochem. J. (1984) **224**, 559–568
Printed in Great Britain

Proteins of the kidney microvillar membrane

Effects of monensin, vinblastine, swainsonine and glucosamine on the processing and assembly of endopeptidase-24.11 and dipeptidyl peptidase IV in pig kidney slices

John R. STEWART and A. John KENNY*
MRC Membrane Peptidase Research Group, Department of Biochemistry, University of Leeds, Leeds LS2 9JT, U.K.

(*Received 3 May 1984/Accepted 20 August 1984*)

The effects of various inhibitors were studied on the biogenesis of endopeptidase-24.11 (EC 3.4.24.11) and dipeptidyl peptidase IV (EC 3.4.14.5) in slices of renal cortex, from piglets of the Yucatan strain, maintained in organ culture. These microvillar peptidases were synthesized within membrane compartments and underwent glycosylation to yield high-mannose and complex forms [the preceding paper, Stewart & Kenny (1984) *Biochem. J.* **224**, 549–558]. Monensin caused very gross ultrastructural changes in the proximal-tubular cells, resulting from distension of the Golgi sacs. It blocked the processing of the high-mannose to the complex glycosylated forms of the peptidases and prevented their assembly in the microvillar membrane. Swainsonine, an inhibitor of α-mannosidase II, generated new 'hybrid' forms of the proteins, intermediate in M_r between the high-mannose and the complex forms, but did not prevent assembly of the hybrid forms in microvilli. Vinblastine, an agent that affects microtubules, delayed, but did not abolish, either the processing or the transport to microvilli. Glucosamine interfered with the initial glycosylation reactions and generated heterogeneous sets of partially glycosylated polypeptides of lower M_r than the high-mannose forms. These results are discussed in relation to the site and mechanism of glycosylation and the involvement of the Golgi complex and microtubules in the biogenesis of these membrane peptidases.

In the preceding paper (Stewart & Kenny, 1984) we reported on the biosynthesis of four kidney microvillar peptidases studied in organ culture of pig renal-cortex slices. We showed that endopeptidase-24.11 (EC 3.4.24.11), depeptidyl peptidase IV (EC 3.4.14.5), aminopeptidase N (EC 3.4.11.2) and aminopeptidase A (EC 3.4.11.7) were detectable as labelled precursors after pulse–chase experiments with [^{35}S]methionine. In each case the form recognized by immunoprecipitation immediately after the pulse was of lower M_r than that previously defined for the same enzymes when purified from pig kidney. These precursors were susceptible to endoglycosidase-H (EC 3.2.1.96) and were therefore designated 'high-mannose forms'.

Abbreviations used: endoglycosidase-H, endo-β-*N*-acetylglucosaminidase-H; SDS, sodium dodecyl sulphate.

* To whom requests for reprints should be directed.

After 30–60min, another form was generated that, in each case, corresponded exactly in apparent subunit M_r with the purified enzyme and which was no longer sensitive to endoglycosidase-H. These forms were therefore considered to have been subjected to complex glycosylation. Moreover, the intensities of the labelled bands on the fluorographs suggested that the first form was the precursor of the second. Both forms were found in the Mg^{2+} pellet, which contains intracellular and basolateral membranes, but relatively few microvilli. When microvillar fractions were examined, the only labelled forms corresponded in M_r to the larger precursors seen in the Mg^{2+} pellet. In the case of one peptidase, dipeptidyl peptidase IV, we showed that the progress curves for the immunoprecipitated radioactivity were consistent with the transport of the precursor from intracellular membranes to microvilli and that this process required about 1.5h. At no time were precursors found in the cytosolic fraction. Thus the biogenesis

of these peptidases occurring within membrane-bound systems and the progression from high-mannose to complex glycosylated forms proceeded in a fashion similar to that reported for brush-border hydrolases studied in organ culture of the intestine (Danielsen, 1982; Danielsen *et al.*, 1983*a*).

In the present paper we have explored the use of several inhibitors capable of illuminating not only the role of the Golgi complex (monensin) and microtubules (vinblastine) but also (in the case of swainsonine and glucosamine) supporting the proposed steps of glycosylation of these proteins.

Experimental

Materials

Monensin was donated by Dr. E. M. Danielsen, Panum Institute, University of Copenhagen, Copenhagen, Denmark. Swainsonine was kindly donated by Dr. P. R. Dorling, School of Veterinary Studies, Murdoch University, Murdoch, Western Australia, Australia. Vinblastine, colchicine, tunicamycin, bacitracin, and glucosamine were purchased from Sigma. All other materials were as described in the preceding paper (Stewart & Kenny, 1984).

Methods

The preparation of slices of renal cortex from Yucatan-piglet kidneys, the culture conditions, pulse–chase experiments with [^{35}S]methionine, endoglycosidase H treatment and subsequent analyses of the immunoprecipitated proteins were also as decribed in the preceding paper (Stewart & Kenny, 1984). The details of the experiments employing inhibitors in the organ culture medium are given in the Results section. None of the inhibitors affected the distribution of the marker enzymes in the subcellular fractionation.

Results

Effect of monensin on the ultrastructure of kidney cortex

When slices of piglet renal cortex were incubated in the presence of 10 μM-monensin, the epithelial cells of the proximal tubule underwent a series of morphological changes. After 60 min, an increase in the size (and probably number) of the apical vacuoles was visible in electron micrographs compared with control slices incubated for 120 min without monensin (Fig. 1*b* compared with Fig. 1*a*). After 120 min most of the cytoplasm, from base to apex of the cell, was occupied with distended smooth-surfaced vacuoles. In other respects, e.g. the shape of microvilli, the integrity of the nuclear membrane and the form of mitochondria, the morphology was unaffected.

In Fig. 2, part of the Golgi complex is shown after 60 min incubation under control conditions (Fig. 2*a*) and in the presence of 10 μM-monensin (Fig. 2*b*). In the latter, one of the cisternae appears to be distended and to have formed multiple small vesicles. Adjacent to these are numerous smooth-surfaced clear vacuoles that have the same diameter as those seen in the apical cytoplasm in Fig. 1(*b*). This appearance is consistent with the reported effects of monensin on the morphology of the Golgi complex in producing vesiculation of the Golgi complex (Tartakoff, 1982).

Effect of monensin on the processing and transport of endopeptidase-24.11 and dipeptidyl peptidase IV

In these experiments piglet kidney slices were preincubated with 10 μM-monensin for 60 min before adding [^{35}S]methionine and incubating for a further 60 min. After homogenization, detergent treatment and immunoprecipitation, fluorographs were prepared of gels after SDS/polyacrylamide-gel electrophoresis. These are shown in Fig. 3. Only one of the two radioactive polypeptides normally observed after 60 min of labelling [see the preceding paper (Stewart & Kenny, 1984)] was seen in the immunoprecipitates of each enzyme. For endopeptidase-24.11 it was a polypeptide of M_r 88000 that was partially sensitive to treatment with endoglycosidase-H. Similarly, in the case of dipeptidyl peptidase IV, monensin prevented the appearance of the 130 kDa complex glycosylated polypeptide normally observed. Again, the smaller polypeptide (115 kDa) was partially sensitive to endoglycosidase-H. Thus monensin halted the normal biogenesis of both peptidases at the stage of the high-mannose (endoglycosidase H-sensitive) forms.

A further experiment was performed to investigate the effect of monensin on the transport of the labelled precursors from intracellular membranes present in the Mg^{2+} pellet to the apical plasma membrane purified in the microvillar fraction. After incubation for 1 h with monensin the slices were pulsed for 15 min with [^{35}S]methionine and chased for 2 h (Fig. 4). In the absence of monensin, the Mg^{2+} pellet contained both precursor forms (M_r 88000 and 93000) of endopeptidase-24.11 and the 115000- and 130000-M_r forms of dipeptidyl peptidase IV; all were visible at 90 and 120 min of the chase period. After 2 h the 93 kDa and 130 kDa bands were also found in the microvillar fraction. However, in the slices treated with monensin, only the low-M_r high-mannose forms were seen in the Mg^{2+} pellet, and no radioactive form was visible in the microvillar pellet. Thus monensin not only

prevented complex glycosylation but also blocked transport to the apical membrane.

Effect of vinblastine and colchicine on processing and transport of endopeptidase-24.11 and dipeptidyl peptidase IV

Piglet kidney slices were preincubated for 1h with vinblastine (20μg/ml) before initiating a 15min pulse with [^{35}S]methionine, followed by a 2h chase period. Mg^{2+}-pellet and microvillar fractions were prepared from these and from control slices (Fig. 5). In the control slices endopeptidase-24.11 was synthesized, processed and transported normally. Vinblastine increased the incorporation of radioactivity approx. 2-fold, but delayed processing and transport. In the Mg^{2+} pellet the high-mannose form predominated at 60 and 120min. No labelled form appeared in the microvillar fraction after 60min, and even at 120min the band was weak and diffuse compared with that in the control. Dipeptidyl peptidase IV was similarly affected: more radioactivity was incorporated, but the processing of the high-mannose form was slowed and no radioactive bands could be demonstrated in the microvillar fraction, even after 120min of chase.

An experiment was performed with 25μM-colchicine in place of vinblastine. After 2h incubation with colchicine the slices were pulsed with [^{35}S]methionine and chased for a period of 2h, colchicine being present throughout. The treated slices did not differ from those of the control, the labelled forms of both peptidases being detected in the microvillar membrane after 90min of the chase period. Electron micrographs of slices treated with vinblastine or colchicine revealed no abnormalities.

Effect of swainsonine on the processing of endopeptidase-24.11 and dipeptidyl peptidase IV

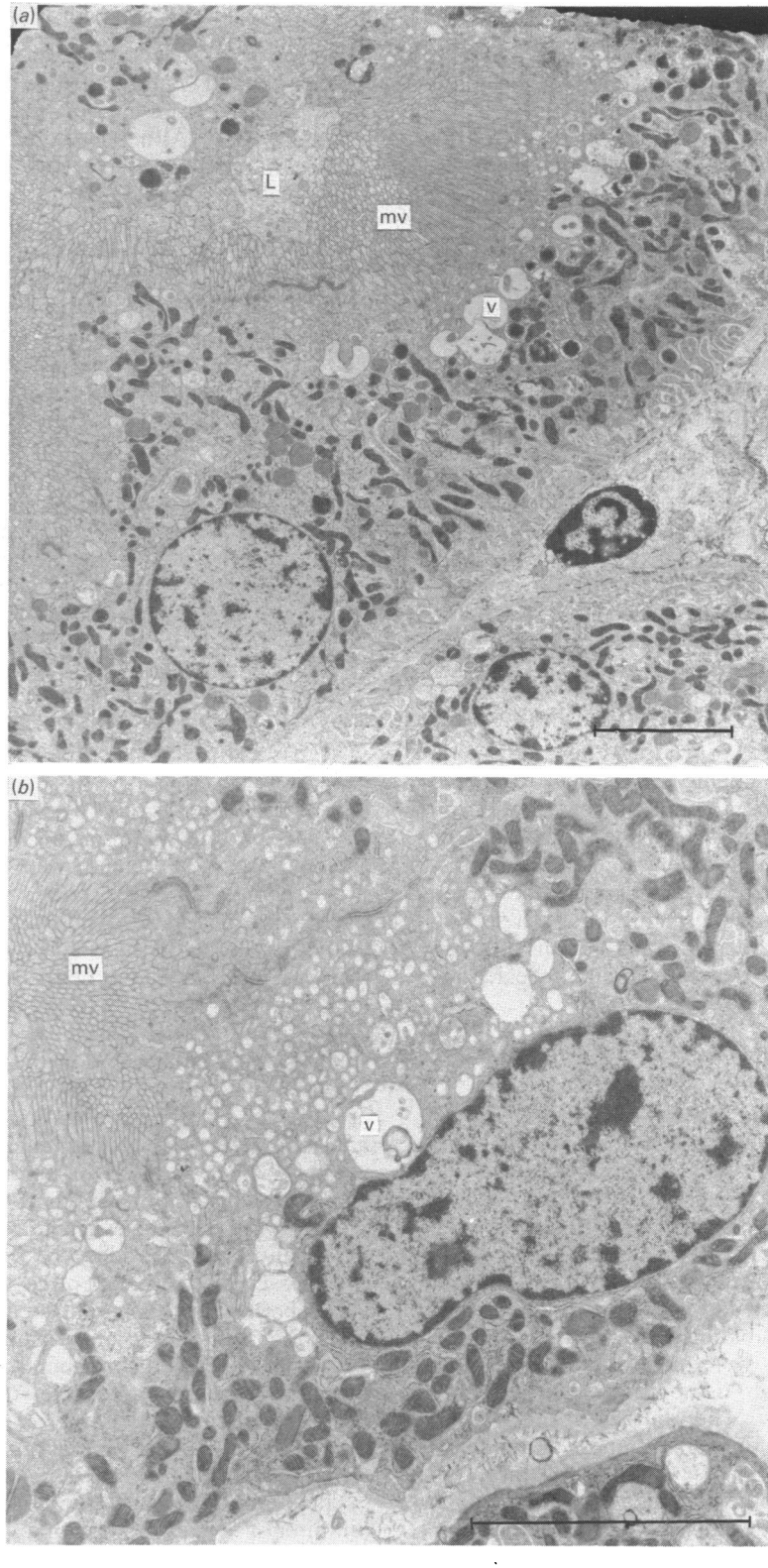
Swainsonine is an alkaloid that inhibits α-mannosidase II, one of the enzymes involved in the trimming of the high-mannose chains within the Golgi complex (Tulsiani *et al.*, 1982). Its effect on the biogenesis of two microvillar peptidases was first examined in an experiment in which kidney slices were preincubated for 60min with swainsonine (20μg/ml) before adding [^{35}S]methionine and continuing the incubation for a further 60min. Radioactive forms were precipitated from the detergent-solubilized tissue from control and experimental slices. The untreated slices contained, as expected, the high-mannose and mature forms of both peptidases (Fig. 6). Swainsonine treatment generated new forms of enzymes. Most of the radioactivity in endopeptidase-24.11 migrated as a band of about 90kDa, i.e. intermediate in M_r between the 88000- and 93000-M_r forms, though the 88kDa form was just discernible. Dipeptidyl peptidase IV was similarly affected, most of the radioactivity being associated with a polypeptide of M_r 120000, with only a small amount being present in the 115kDa high-mannose band. Although larger in size than the initial high-mannose precursors, these intermediate forms were sensitive to treatment with endoglycosidase H (results not shown).

The effect of swainsonine on the transport of these peptidases to the microvillar membrane was studied in a further experiment in which the kidney slices were pulse-chased and then fractionated into Mg^{2+} pellet and microvillar fractions (Fig. 7). The Mg^{2+} pellet revealed the same radioactive forms observed in the first experiment, with most of the radioactivity present in the novel 90 and 120kDa polypeptides at 90 and 120min of the chase period. The microvillar fraction also contained radioactive forms that were not of the mature size, but of the intermediate values. Thus, unlike monensin and vinblastine, swainsonine did not impair the transport of the precursors to the microvillar membrane, nor did it affect the ultrastructure of the tissue.

Effect of tunicamycin, bacitracin and glucosamine on the processing of endopeptidase-24.11 and dipeptidyl peptidase IV

A series of experiments designed to study the influence of three inhibitors on *N*-glycosylation by the endoplasmic reticulum were performed. Preincubation (3.5h with either tunicamycin or bacitracin, each at 20μg/ml) inhibited incorporation of radioactivity into tissue proteins by 60 and 100% respectively and in neither experiment were any immunoprecipitable forms of endopeptidase-24.11 or dipeptidyl peptidase IV detected by fluorography. The addition of leupeptin (10μg/ml), as an inhibitor of intracellular proteinases, in the experiment with tunicamycin did not alter the result. However, tunicamycin did not wholly abolish synthesis of microvillar enzymes, since two labelled polypeptides of M_r 140000 and 115000 were detected when immunoprecipitated by an anti-aminopeptidase N serum (results not shown). The latter polypeptide is identical with that reported for this peptidase in similar experiments in intestinal explants (Danielsen & Cowell, 1984) and appears to correspond to the size of the primary translation product of this enzyme in a cell-free system (Danielsen *et al.*, 1982), whereas the former corresponds to that processed by microsomal membranes (Danielsen *et al.*, 1983*b*).

Glucosamine has been shown to be a reversible inhibitor of *N*-glycosylation of a viral coat protein (Schwarz *et al.*, 1977; Koch *et al.*, 1979; Datema & Schwarz, 1979). In one experiment (results not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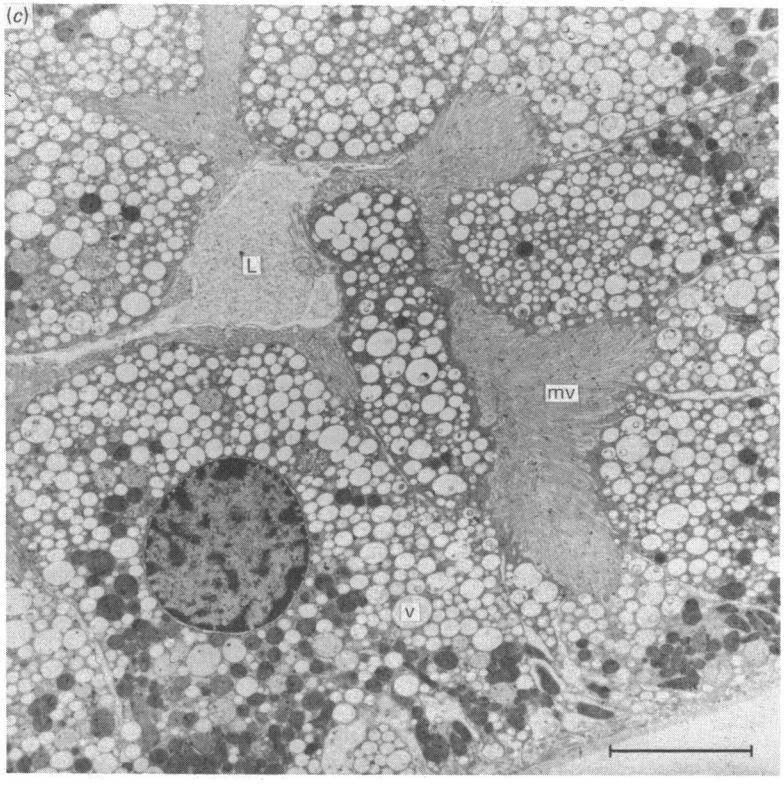

Fig. 1. *Effect of monensin on the ultrastructure of kidney proximal-tubule cells*
(*a*) Control kidney slice incubated for 2h under standard conditions. (*b* and *c*) Kidney slices incubated in the presence of 10 μM-monensin for 60min (*b*) and 120min (*c*). Note the formation of numerous vacuoles in the monensin-treated slice. The bar represents 5 μm. Abbreviations used: mv, microvilli; L, tubule lumen; v, vacuole.

shown) piglet kidney slices were preincubated with 10mM-glucosamine for 60min before addition of [^{35}S]methionine and incubation for a further 60min. A total membrane fraction was prepared. In these conditions, glucosamine reduced the radioactivity in the bands identified as the high-mannose forms of endopeptidase-24.11 and dipeptidyl peptidase IV and prevented the formation of the complex forms. Additionally, a series of radioactive bands extending from M_r 115000 to M_r 88000 were visible in the case of dipeptidyl peptidase IV, and a blurred band migrating ahead of the 88kDa polypeptide was seen in the case of endopeptidase-24.11. The mature forms of the enzymes were not observed.

Discussion

Sites of action of the inhibitors

Endoplasmic reticulum. Proteins destined for the plasma membrane are synthesized by membrane-bound polyribosomes, and microvillar membrane hydrolases are no exception to this rule. None of the labelled forms in our studies on four kidney microvillar peptidases [see the preceding paper (Stewart & Kenny, 1984)] nor of the group of intestinal microvillar hydrolases investigated by Danielsen and co-workers (Danielsen, 1982; Danielsen *et al.*, 1983*a*) have been identified in the cytosol. The earliest form was already glycosylated as a high-mannose glycoprotein, and this processing is therefore likely to be a co-translational event. Asparagine-linked carbohydrate chains are assembled by transfer of lipid-linked precursor oligosaccharide chains (for review, see Hubbard & Ivatt, 1981). These high-mannose chains initially have the structure Asn-$(GlcNAc)_2$-Man_9-Glc_3, but the glucose residues are rapidly removed after transfer, and they are sensitive to cleavage by endoglycosidase H at the GlcNAc–GlcNAc linkage (Tarentino & Maley, 1974). Tunicamycin acts at an early stage in the assembly of the lipid-linked oligosaccharide by blocking the glycosylation of dolichol phosphate by *N*-acetylglucosamine (Lehle & Tanner, 1976; Keller *et al.*, 1979; Heifetz *et al.*,

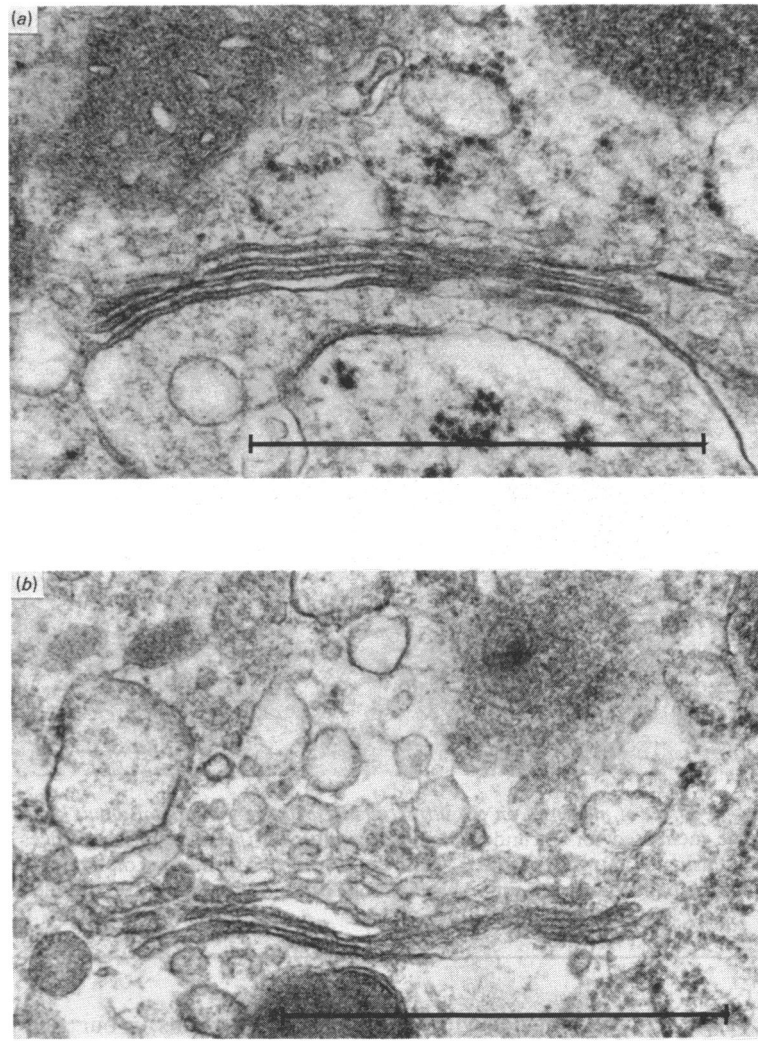

Fig. 2. *Effect of monensin on the Golgi complex*
(*a*) Control slice incubated for 1 h. Four cisternae can be seen without any associated vesicles. (*b*) Slice incubated in 10 μM-monensin. Note the vesicles closely associated with the Golgi stack, the largest of which are of similar diameter to those in Fig. 1(*b*). The bar represents 1 μm in (*a*) and (*b*).

1979). But as noted by Schwaiger & Tanner (1979) both this inhibitor and bacitracin prevented any incorporation of radioactivity into the two peptidases.

Golgi complex. The role of the Golgi complex in processing and transporting secretory, lysosomal and membrane proteins is now being elucidated (for review, see Farquhar & Palade, 1981). It is clear that the conversion of the high-mannose to the complex glycosylated form of glycoproteins occurs in the Golgi, as does *O*-glycosylation. Within the stack of Golgi cisternae, specialization in function is known to exist. The conversion of the Asn-$(GlcNAc)_2Man_9$ chains into complex oligosaccharides requires the stepwise removal of the mannose residues: Mannosidase I converts R-Man_9 into R-Man_5 [where R is Asn-$(GlcNAc)_2$]; *N*-acetylglucosamine transferase I adds a GlcNAc residue to the unbranched antenna before mannosidase II converts R-Man_5-GlcNAc into R-Man_3-GlcNAc. The subsequent steps involve *N*-acetylglucosaminotransferase II, galactosyltransferase and sialyltransferase. Dunphy & Rothman (1983) have succeeded in resolving Golgi membranes that are involved in the glycosylation reactions. One fraction catalysing four steps (mannosidase I to *N*-

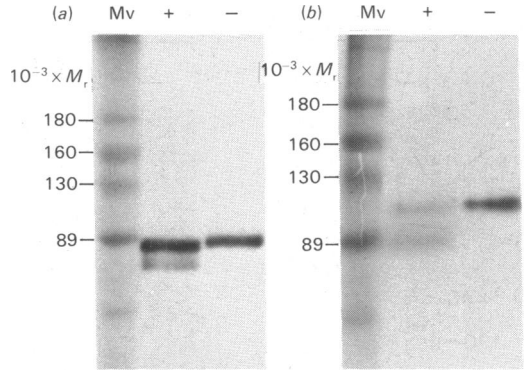

Fig. 3. *Effect of monensin on glycosylation of endopeptidase-24.11 and dipeptidyl peptidase IV*

Slices were cultured for 2h in the presence of monensin, with [^{35}S]methionine added for the last hour. After culture, a Triton X-100 extract of total membrane protein was prepared, the enzymes were purified by immunoprecipitation and treated with endoglycosidase-H. The latter were subjected to SDS/polyacrylamide-gel electrophoresis followed by fluorography. + and − indicate with and without endoglycosidase-H treatment. (*a*) Endopeptidase-24.11; exposure time 12 days. (*b*) Dipeptidyl peptidase IV; exposure time 10 days. Abbreviation used: Mv, ^{125}I-labelled pig kidney microvillar proteins.

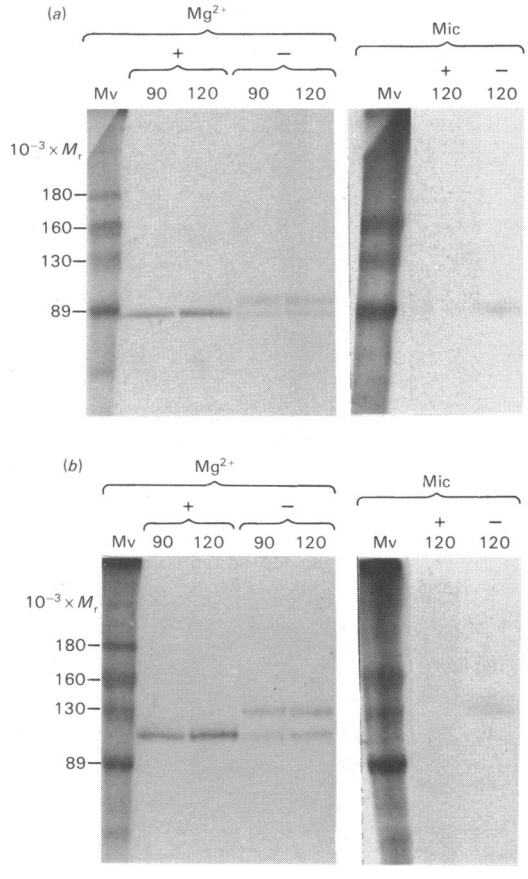

Fig. 4. *Effect of monensin on transport of endopeptidase-24.11 and dipeptidyl peptidase IV*

Slices were pre-treated with monensin for 1 h before pulse–chase labelling with [^{35}S]methionine. After culture, both enzymes were immunoprecipitated from a solubilized Mg^{2+}-precipitated membrane fraction (Mg^{2+}) and a solubilized microvillar fraction (Mic). The purified enzymes were subjected to SDS/polyacrylamide-gel electrophoresis followed by fluorography. + and − indicate in the presence or absence of monensin. The chase time (min) is indicated above the gel tracks. (*a*) Endopeptidase-24.11; (*b*) dipeptidyl peptidase IV. Exposure times were 11 days in the case of Mg^{2+}-precipitated membrane fractions and 30 days in the case of microvillar membrane fractions. Abbreviation used: Mv, ^{125}I-labelled pig kidney microvillar proteins.

acetylglucosaminotransferase II) could be resolved from a fraction catalysing the last two (galactosyltransferase and sialyltransferase), which are known to be associated with *trans* Golgi membranes. It was suggested, therefore, that the early steps, including mannosidase II, are located in some or all of the *cis* Golgi membranes. Swainsonine is an indolizidinitriol alkaloid isolated from *Swainsona canescens* that inhibits α-mannosidase (Colegate *et al.*, 1979) and which can block glycoprotein processing in cultured cells (Elbein, 1981), including a viral coat protein (Elbein *et al.*, 1982). Its site of action is therefore likely to be on mannosidase II located in the *cis* Golgi cisternae. When kidney slices were cultured in the presence of swainsonine, unusual forms of the peptidases were generated, which were intermediate in M_r between the high-mannose and mature forms, but which were transported to the microvillar membrane. These novel or hybrid forms retained their sensitivity to endoglycosidase H and hence may be designated 'high-mannose forms'. The increase in M_r over that of the normal high-mannose forms may be attributable to *O*-glycosylation. Danielsen *et al.* (1983*d*) observed that swainsonine in intestinal organ culture yielded a 160kDa polypeptide that, unlike the normal 166kDa mature enzyme, retained sensitivity to endoglycosidase-H.

Monensin is a carboxylic ionophore with a greater specificity for Na^+ than for other univalent cations (Pressman, 1976), such that cells lose K^+ and gain Na^+. It blocks intracellular transport of secretory proteins by an effect on the Golgi complex (Tartakoff & Vassalli, 1977, 1979). A

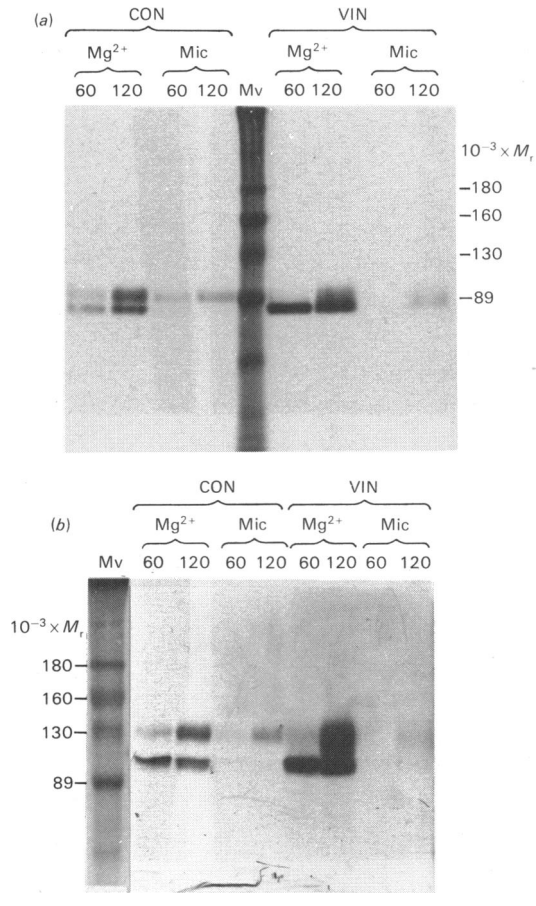

Fig. 5. *Effect of vinblastine on transport of endopeptidase-24.11 and dipeptidyl peptidase IV*

Slices were pretreated with vinblastine for 1 h before pulse–chase labelling with [^{35}S]methionine. After culture, both enzymes were immunoprecipitated from a solubilized Mg^{2+}-precipitated membrane fraction (Mg^{2+}) and a solubilized microvillar membrane fraction (Mic). The purified enzymes were subjected to SDS/polyacrylamide-gel electrophoresis followed by fluorography. CON and VIN indicate control and vinblastine-treated slices respectively. The chase time (min) is shown above the gel tracks. (*a*) Endopeptidase-24.11; (*b*) dipeptidyl peptidase IV. Exposure times in both instances were 10 days. Abbreviation used: Mv, ^{125}I-labelled pig kidney microvillar proteins.

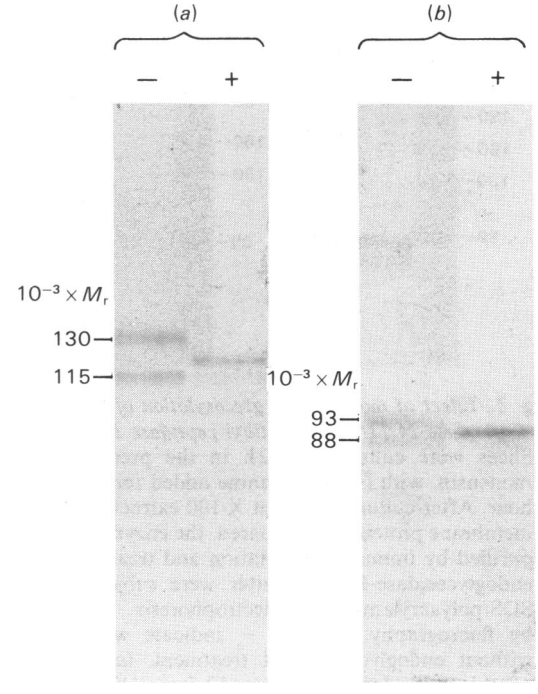

Fig. 6. *Effect of swainsonine on the biosynthesis of endopeptidase-24.11 and dipeptidyl peptidase IV*

Slices were preincubated for 1 h with swainsonine (20 μg/ml) before adding [^{35}S]methionine and incubating for a further 60 min. Immunoprecipitates were prepared from a total membrane fraction after solubilization with Triton X-100; (*a*) dipeptidyl peptidase IV; (*b*) endopeptidase-24.11. + and − indicate the presence and absence of swainsonine.

recent electron-microscopic/immunocytochemical study (Griffiths *et al.*, 1983; Quinn *et al.*, 1983), on the effect of monensin on the transport of Semliki-Forest-virus capsids, led to the proposal that three compartments may be delineated. The functional block by monensin caused the accumulation of capsids in swollen Golgi cisternae that were defined as *medial* rather than *cis* or *trans*. Our experiments confirmed that monensin produced a dramatic alteration in the morphology of the proximal-tubule cells. It also halted the processing of the peptidases at a precursor stage and prevented transport to the microvillar membrane. The 88 kDa and 115 kDa polypeptides were only partially sensitive to endoglycosidase-H, probably indicating that some preliminary processing had occurred in the *cis* Golgi cisternae. These results agree with those reported for aminopeptidases N and A, dipeptidyl peptidase IV, sucrase–isomaltase and maltase–glucoamylase in intestinal explants (Danielsen *et al.*, 1983*c*).

Microtubules. The two agents used, colchicine and vinblastine, are both capable of disorganizing microtubules. Colchicine binds in a temperature- and time-dependent fashion to tubulin dimers and thereby prevents the assembly of microtubules. Vinblastine also binds to tubulin and is capable of causing dissolution of microtubules, sometimes with the formation of crystalline complexes (Wilson, 1975). In our experiments, 25 μM-colchicine

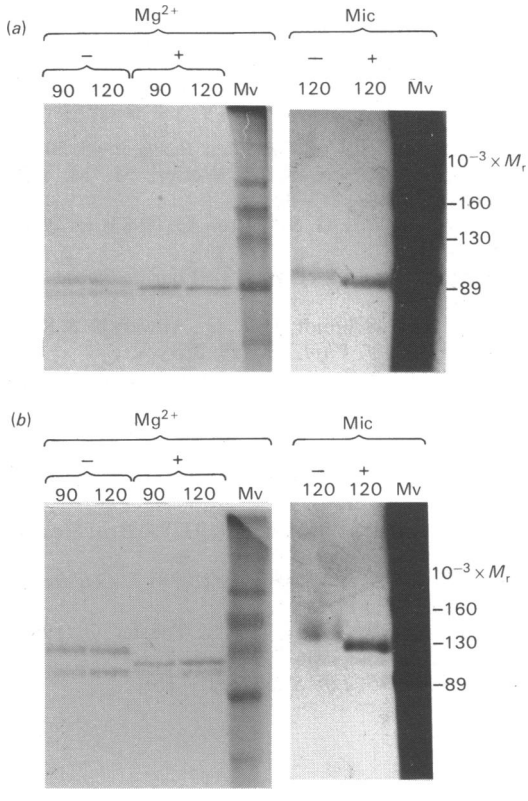

Fig. 7. *Effect of swainsonine on the transport of endopeptidase-24.11 and dipeptidyl peptidase IV*

Slices were pretreated with swainsonine 20 μg/ml for 1 h before pulse–chase labelling with [^{35}S]-methionine, After culture, both enzymes were immunoprecipitated from a solubilized Mg^{2+}-precipitated membrane fraction (Mg^{2+}) and a solubilized microvillar membrane fraction (Mic). The purified enzymes were subjected to SDS/polyacrylamide-gel electrophoresis followed by fluorography. + and – indicate the presence or absence of swainsonine. The length of chase (min) is indicated above the gel tracks. (*a*) Endopeptidase-24.11; (*b*) dipeptidyl peptidase IV. Exposure times were 10 days in the case of Mg^{2+}-precipitated membrane fractions and 30 days in the case of microvillar membrane fraction. Abbreviation used: Mv, ^{125}I-labelled pig kidney microvillar proteins.

had no effect on the biosynthesis or assembly of the two peptidases in the microvillar membrane. However, Danielsen *et al.* (1983*c*) observed a colchicine effect on the transport of several microvillar hydrolases to the intestinal brush border. This possibly reflects the higher concentration (125 μM) and longer preincubation period (5 h) in their experiments. Vinblastine (22 μM) had a marked effect in our system when preincubated for 1 h before the radioactive pulse. It produces an unexpected stimulation of incorporation of radioactivity into the slice protein and delayed both processing of the high-mannose to the complex forms and transport of the latter to the microvillar membrane. Although these results are consistent with an effect on microtubules, vinblastine, like colchicine, may produce effects unrelated to disruption of microtubules (Wilson, 1975). Nevertheless, there are many reports on the effect of antimicrotubular agents on intracellular transport of secretory proteins, and some have implicated the Golgi complex in these effects, since fragmentation of the cisternae have been reported. In a recent study, Pavelka & Ellinger (1983) suggest that microtubules may exert a regulatory function in the organization of the Golgi complex.

Biogenesis and intracellular transport of endopeptidase-24.11 and dipeptidyl peptidase IV

The earliest stage in the biogenesis of these peptidases at which an inhibitor effect was detected related to the initial lipid-linked *N*-glycosylation, occurring co-translationally, in the endoplasmic reticulum. Monensin and swainsonine inhibited processing in the Golgi cisternae, the former arresting biogenesis at the high-mannose form and preventing onward transport through the *trans* Golgi to the microvillar membrane; the latter by producing novel forms of the peptidases that were not apparently hindered in their progress to the microvillar membrane. The effect of vinblastine may also have been exerted at the Golgi complex. Processing of the high-mannose to the complex form was delayed, but not prevented. So, too, was the assembly of the peptidases in the microvillar membrane. Quaroni *et al.* (1979) studied the effects of colchicine *in vivo* on the transport of [^{3}H]fucose-labelled glycoproteins in the rat intestine. They concluded that colchicine affected the intracellular transport of glycoproteins to the microvillus membrane and that the normal route from the Golgi complex to microvillus was via the basolateral membrane. Neither our results, nor those of Danielsen *et al.* (1983*c*) throw any light on this question.

We thank the Medical Research Council for financial support, Mr. Douglas Kershaw for the electron microscopy and the University of Leeds for the award of a Studentship to J. R. S.

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