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Effect of Monensin on Ricin and Fluid Phase Transport in Polarized MDCK Cells

Elisabeth Løkken Melby, Kristian Prydz, Sjur Olsnes, and Kirsten Sandvig

Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, 0310 Oslo 3, Norway

Abstract The effect of monensin on endocytosis, transcytosis, recycling and transport to the Golgi apparatus in filter-grown Madin-Darby canine kidney (MDCK) cells was investigated using ^{125}I-labeled ricin as a marker for membrane transport, and horseradish peroxidase (HRP) as a marker for fluid phase transport. Monensin (10 μM) stimulated transcytosis of both markers about 3-fold in the basolateral to apical direction. Transcytosis of HRP in the opposite direction, apical to basolateral, was reduced to approximately 50% of the control by monensin, whereas that of ricin was slightly increased. Recycling of markers endocytosed from the apical surface was reduced in the presence of monensin and there was an increased accumulation of both ricin and HRP in the cells. Transport of ricin to the Golgi apparatus increased to the same extent as the increase in intracellular accumulation. No change in recycling or accumulation was observed with monensin when the markers were added basolaterally, but transport of ricin to the Golgi apparatus increased almost 3-fold. Our results indicate that basolateral to apical transcytosis is increased in the absence of low endosomal pH, and they suggest that apical to basolateral transcytosis of a membrane-bound marker (ricin) is affected by monensin differently from that of a fluid phase marker (HRP).

Key words: Golgi, transcytosis, endocytosis, epithelial cells, horseradish peroxidase

Fluid and membrane-associated molecules taken up by endocytosis in eukaryotic cells may follow a number of intracellular pathways (Wileman et al., 1985). The first sorting site seems to be the peripheral early endosome. In polarized cells, which have two distinct plasma membrane domains and distinct classes of early endosomes (Bomsel et al., 1989; Parton et al., 1989), endocytosed molecules may be recycled back to the plasma membrane, transported deeper into the cell towards the late endosomes, lysosomes, the trans-Golgi network (TGN), or they may be transcytosed (Bomsel et al., 1989; van Deurs et al., 1990; Brändli et al., 1990; Pesonen et al., 1984b; Mostov and Deitcher, 1986; Maratos-Flier et al., 1987). Mannose-6-phosphate receptors and the transferrin receptor are examples of molecules that recycle partly via the trans Golgi network (Duncan and Kornfeld, 1988; Goda and Pfeffer, 1988; Wileman et al., 1985; Snider and Rogers, 1985). Also, the plant toxin ricin that binds to a variety of glycoproteins and glycolipids at the cell surface (for review, see van Deurs et al., 1987, 1990; Sandvig et al., 1986) and Shiga toxin that binds to certain glycolipids (Sandvig et al., 1989, 1991a; Lindberg et al., 1987) are transported to the Golgi apparatus.

Low intravesicular pH is an important requirement for many transport processes. Alkalinization of intracellular vesicles by carboxylic ionophores and acidotropic amines may therefore perturb sorting processes and alter intracellular traffic. For instance, recycling of a number of receptors (e.g. the receptor for epidermal growth factor and low density lipoprotein receptor) (Gladhaug and Christoffersen, 1988; Tartakoff, 1983) is inhibited in the presence of the ionophore monensin. Sorting of transcytosed molecules seems to occur in the endosomes (Pesonen et al., 1984a,b), presumably already in the early endosomes (Bomsel et al., 1989; Parton et al., 1989). Since the internal milieu of endosomal compartments normally is acidic, low intravesicular pH could be an important factor in the regulation of transcytosis. It has been shown that monensin (10 μM) and ammonium chloride (10 mM) inhibit transcytosis of vesicular stomatitis virus G protein implanted into the apical plasma membrane of Madin-Darby canine kidney (MDCK) epithelial cells (Pesonen and Simons, 1983). Furthermore, 50 μM monensin was

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Address reprint requests to Kirsten Sandvig at the address given above.

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recently reported to reduce transcytosis of angiotensin II across cultured endothelial cell layers (Mineo et al., 1990). On the other hand, monensin had no effect on transcytosis of HRP from the apical to the basolateral surface in CaCo-2 cells (Heyman et al., 1990).

To investigate the role of low intravesicular pH in transepithelial transport and endocytosis in MDCK cells, we have studied the effect of monensin (10 μM) on internalization, transcytosis, and recycling of the fluid phase marker horseradish peroxidase (HRP) and the membrane marker ricin (for review, see van Deurs et al., 1989). Also, we have investigated whether or not monensin affects transport of ricin to the Golgi apparatus. Ricin binds to glycoproteins and glycolipids with terminal galactose residues, and the dissociation from the binding sites is low, even at pH values obtained in endosomes (Sandvig et al., 1976). Previous work showed that ricin was transcytosed and transported to the trans Golgi network (TGN) from both poles of filter-grown MDCK cells (van Deurs et al., 1990). The cytotoxic effect of ricin consists in inhibition of protein synthesis by the enzymatically active A-chain which enters the cytosol (Olsnes and Pihl, 1973). Several lines of evidence suggest that this entry occurs after transfer of ricin to the Golgi apparatus (Sandvig et al., 1986; van Deurs et al., 1986; Voule and Colombatti, 1987), and we have therefore also studied to what extent monensin affects the intoxication of the cells.

MATERIALS AND METHODS

Materials

Ricin was purified as described earlier (Olsnes and Pihl, 1973). ^{125}I-labeled ricin was prepared according to the method described by Fraker and Speck (1978). Na^{125}I and [^{3}H]leucine were obtained from the Radiochemical Center, Amersham, UK. Horseradish peroxidase type VI (HRP) was obtained from Sigma Chemical Co., St. Louis, Mo. Nycodenz was obtained from NYCOMED, Oslo, Norway.

Cell Culture

Madin-Darby Canine Kidney (MDCK) epithelial cells, strain I (high resistance), were grown and plated on polycarbonate filters (Costar, Badhoevedorp, The Netherlands; cat. no. 3412) in Dulbecco's modified Eagle's medium (Flow Laboratories, Irvine, Scotland) containing 5% fetal calf serum and 2 mM glutamine (Flow Laboratories or Gibco Ltd., Paisley, Scotland) as described by van Deurs et al. (1990) or in MEM with Earle's salt (Gibco) containing 10% fetal calf serum and 2 mM glutamine, as described (Bomsel et al., 1989). Essentially the same results were obtained with either culture condition. In some experiments the cells were grown and plated in medium containing penicillin (100 U/ml, Gibco) and streptomycin (100 μg/ml, Gibco). All cultures used for experiments had a transepithelial resistance of at least 1000 ohm × cm^2.

Binding, Endocytosis, and Transcytosis of ^{125}I-labeled Ricin

The cells were briefly washed with a Hepes buffered medium and the monolayers were then incubated as described in the legends to the tables and figures. The medium contained 0.2–0.6% bovine serum albumin. In some experiments, HRP (2 mg/ml) was added together with ^{125}I-labeled ricin. Essentially the same results were obtained with and without addition of HRP. After the incubation, some of the monolayers were washed in medium at 4°C to determine the total amount of cell-associated ricin. Other monolayers were used to determine the amount of endocytosed ricin as the amount of lactose-resistant ricin associated with the cells (Sandvig and Olsnes, 1979). The amount of transcytosed ricin was determined as the amount of tricloroacetic acid-precipitable radioactivity that had been transported to the opposite pole of the cells (van Deurs et al., 1990).

Measurement of Protein Synthesis Inhibition

The cells were briefly washed with a Hepes buffered medium and incubated as described in the legend to Figure 5 in the same medium with the addition of 0.6% bovine serum albumin. After the incubation, the toxin-containing medium was replaced by medium containing 1 μCi/ml [^{3}H]leucine (no unlabeled leucine). The cells were allowed to incorporate [^{3}H]leucine for 20 min at 37°C. Then the medium was removed and 5% trichloroacetic acid was added. Ten minutes later the monolayers were washed twice in trichloroacetic acid. Finally, the filters were cut out of their plastic holders and transferred to counting vials, 4 ml of Opti-flour^{R} was added and the radioactivity was measured. The results are expressed as the percentage of the incorporation in monolayers to which the addition of toxin had

been omitted. The deviations between parallels were less than 10%.

Subcellular Fractionation Studies

The cells were briefly washed in a Hepes-buffered medium with 0.2% bovine serum albumin and incubations were performed as described in the legend to Table IV and Figure 4. Some filters were incubated without ^{125}I-labeled ricin and used for localization of marker enzymes. HRP was added to label endosomes. The subcellular fractionation method here used has been applied to MDCK cells in an earlier study (Sandvig et al., 1991a), where the method is described in detail. In that study we determined the localization in the gradients of early and late endosomes, as well as the Golgi apparatus. In principle, the fractionation was carried out in the following way: After the incubation with ricin and HRP the cells were chilled, cut out of their plastic holders, and they were washed 3 times for 10 min with shaking in cold PBS containing 0.2% bovine serum albumin and 0.1 M lactose. Homogenization buffer (H-buffer: 0.3 M sucrose, 3 mM imidazole, pH 7.4) (0.5 ml) was added to each filter and the cells were scraped off the filters with a rubber policeman. Each experimental value is based on pooled cells from 6 monolayers. After centrifugation for 10 min at 100*g*, 1 ml of H-buffer was added to the pellet and the cells were resuspended and homogenized. Post-nuclear supernatants were prepared by centrifugation of the homogenates at 2,500 rpm for 10 min in 1.5 ml Eppendorf tubes in an Eppendorf 5415 centrifuge. The system used for fractionation of the post-nuclear supernatant (discontinuous gradient sentrifugation) was similar to the one described by Sandberg et al. (1980). Gradients were prepared in SW 40 tubes from 4.5 ml of light solution (1.15 M sucrose, 15 mM CsCl) and 1.5 ml of heavy solution (1.15 M sucrose, 15 mM CsCl, 15% Nycodenz (w/v)), using a Biocomp Gradient Master (Nycomed, Oslo/Norway, angle 74, speed 16, time 2 min 45 sec). A mixture (usually a total volume of 1.5 ml) of the post-nuclear supernatant (5.6 parts) and 2 M sucrose (4.4 parts) was put on top of the gradient. Then, 3 ml of 0.9 M sucrose and finally 1–2 ml of 0.3 M sucrose were added to each tube. The gradients were fractionated after centrifugation for 4 h 30 min at 33,000 rpm and the amounts of ^{125}I-labeled ricin, HRP, and marker enzymes were determined in the fractions.

Analysis of Cell Fractions

The amount of esterase and β-N-acetyl-glucosaminidase was measured according to Beaufay et al. (1974), and the amounts of HRP and UDP-galactose:glycoprotein galactosyl transferase were measured according to Steinman et al. (1976) and Brändli et al. (1988), respectively. The amount of ^{125}I-labeled ricin endocytosed by the cells was calculated from the radioactivity measured in the fractions. The amount of ^{125}I-labeled ricin in the Golgi complex was calculated from the radioactivity measured in fractions corresponding to those containing UDP-galactose:glycoprotein galactosyl transferase.

Quantification of Endocytosis, Transcytosis, and Recycling of HRP

The monolayers were briefly washed with a Hepes buffered medium (0.2% bovine serum albumin) and the cells were incubated as described in the legends to Table II, Table III, and Figure 3. Surface bound HRP was removed by washing at 4°C as described earlier (Bomsel et al., 1989), and endocytosed HRP was extracted during a 30-min incubation with lysis buffer (1% w/v Triton X-100, 0.05% w/v SDS) at 4°C. Transcytosed HRP was measured as the amount that, at the end of the incubation, was present in the medium at the opposite pole (Bomsel et al., 1989; von Bonsdorff et al., 1985). To measure recycling, monolayers that had been loaded with HRP were incubated as described in the legend to Table III before the amount of recycled HRP was determined (Bomsel et al., 1989). After the last incubation, the amount of the enzyme remaining in the cells was determined. HRP was quantified as described above, and volumes of fluid endocytosed, transcytosed, and recycled per filter were estimated according to Bomsel et al. (1989).

RESULTS

Effect of Monensin on Endocytosis and Recycling of Ricin and HRP

We first measured the effect of monensin on the endocytic uptake of ricin. As shown in Table I, monensin-treatment did not affect internalization of ricin from the basolateral surface. However, when ricin was added at the apical pole of the cells, the amount of internalized toxin was higher in the monensin-treated cells than in the control cells. Similar data were obtained when we measured endocytosis of HRP (Table II). Monensin increased the amount of HRP endocy-

TABLE I. Effect of Monensin on Endocytic Uptake of ^{125}I-labeled Ricin*

Addition of toxin	No monensin	Monensin added
	Endocytosed ricin (% of total bound)	
Apical	7.1 ± 1.2	14.6 ± 1.2
Basolateral	27.1 ± 1.7	28.5 ± 4.4

*Filter-grown cells were incubated for 15 min at 37°C with or without monensin (10 μM). ^{125}I-labeled ricin, (18–221 ng/ml; spes. act. 23,600–36,850 cpm/ng), was then added, either to the apical or to the basolateral surface; and the cells were incubated for 1 h at 37°C. The amount of endocytosed ricin was determined as described in Materials and Methods. The data represent average ± SD of 3 experiments.

tosed from the apical pole, whereas the uptake from the basolateral pole was unaffected.

The possibility existed that the monensin-induced increase in accumulation of ricin and HRP added to the apical side could be due to reduced recycling rather than increased endocytosis. To test whether or not monensin affected the process of recycling, the cells were loaded with ^{125}I-labeled ricin or HRP in the presence and in the absence of monensin. The rate of toxin and HRP recycling to the apical surface was then measured. As shown in Figure 1 and Table III, both recycling of ricin and HRP was indeed reduced in monensin-treated cells suggesting that reduced recycling can be responsible for the increased amounts of ricin and HRP associated with the cells in the presence of monensin. In the case of ricin the difference in the rate of recycling may be even greater than demonstrated in Figure 1, since we cannot measure this rate during the removal of surface-bound toxin. In the case of HRP reduced transcytosis to the basolateral side will also contribute to

TABLE II. Effect of Monensin on Endocytic Uptake of HRP*

Addition of HRP	No monensin	Monensin added
	Endocytosed volume[1] (nl)	
Apical	1.1 ± 0.1	1.7 ± 0.3
Basolateral	31.3 ± 0.9	30.4 ± 1.7

*Filter-grown cells were incubated with HRP (2–5 mg/ml) for 1 h at 37°. Then the amounts of endocytosed HRP were determined as described in Materials and Methods. The data represent mean ± SD of 6 experiments.

[1]Volumes were estimated as described in Materials and Methods.

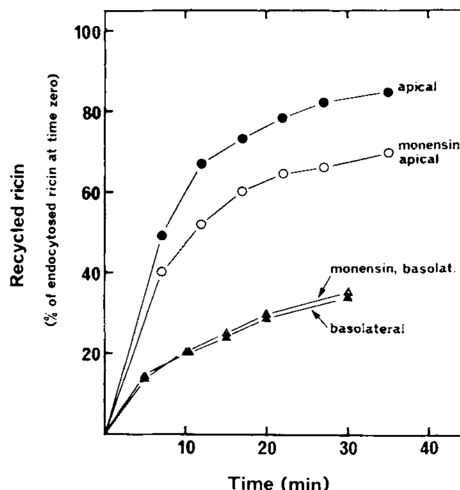

Fig. 1. Effect of monensin on recycling of ricin to the apical and basolateral pole of MDCK cells. Filter grown cells were incubated with and without monensin (10 μM) for 15 min at 37°C. Then ^{125}I-labeled ricin (1 μg/ml; spes. act. 20,140 cpm/ng) was added at the apical or the basolateral pole of the cells and the incubation was continued for another 20 min. The medium was then removed and the cells were incubated with 0.1 M lactose in medium for 8 min at 37°C. They were then washed rapidly three times in the same solution. Some filters were at this point counted to determine the amount of endocytosed toxin, whereas the remaining filters were incubated in the presence of 2 mM lactose for the indicated periods of time before medium containing recycled ricin was removed and fresh medium was added to the same filters. The total amount of ^{125}I-labeled ricin recycled after a given time was calculated from the amounts recycled in the different time intervals and is expressed as the percentage of ricin endocytosed after 20 min. The deviations between parallells were less than 5%.

increased intracellular accumulation of this compound (see below). There was no effect on recycling to the basolateral domain (Fig. 1, Table III).

Effect of Monensin on Transcytosis of Ricin and HRP

We then investigated whether or not monensin had any effect on transcytosis of ^{125}I-ricin and HRP. Cells growing on polycarbonate filters were incubated with and without monensin for 15 min before ricin or HRP was added. The amounts of transcytosed ricin and HRP were measured after 60 min at 37°C. The effects of monensin on transcytosis of ricin and HRP were found to be different. Thus, the amount of transcytosed ricin was increased in the presence of monensin, both when the toxin was added at the apical and the basolateral side of the cells (Fig. 2), the increase being largest upon addition of toxin basolaterally. On the other hand, monen-

TABLE III. Effect of Monensin on Recycling of HRP*

Monensin	−	+	−	+
HRP added	Recycled volume[1]		Volume remaining in the cells[1]	
	nl		nl	
Apically	0.17 ± 0.03	0.13 ± 0.03	0.32 ± 0.04	1.05 ± 0.13
Basolaterally	1.6 ± 0.4	1.7 ± 0.3	10.7 ± 2.3	10.8 ± 1.8

*Filter-grown cells were incubated in the presence or in the absence of monensin (10 μM) for 15 min at 37°C. HRP (2–5 mg/ml) was then added, either at the apical surface or at the basolateral surface, and the incubation was continued for 20 min at 37°C. At this point, the cells were washed as described in Materials and Methods and a second incubation at 37°C in HRP-free medium with and without monensin (10 μM) was performed. After 15 min, the amount of recycled HRP and the amount of HRP remaining in the cells were determined as described in Materials and Methods. The data represent mean ± SD of 5 to 6 experiments.

[1]Volumes were estimated as described in Materials and Methods.

sin reduced the amount of transcytosed HRP from the apical to the basolateral pole (Fig. 3), in spite of the increased amount of HRP accumulated intracellularly in the presence of monensin (Table II). Monensin increased the transcytosis of HRP from the basolateral to the apical pole in a way similar to what was found for ricin (Fig. 3).

Effect of Monensin on Transport of Ricin to the Golgi Complex

Previous data have shown that ricin is transported to the trans-Golgi network (TGN) from both the apical and the basolateral membrane domains of MDCK cells (van Deurs et al., 1990). Transport to the Golgi complex is thought to be an important step preceding the entry of ricin A-chain into the cytosol (Sandvig et al., 1986; van Deurs et al., 1986). To test whether or not transport to the Golgi complex was also affected by monensin-treatment, the cells were incubated with ^{125}I-labeled ricin in the presence or absence of monensin, and Golgi elements were isolated by subcellular fractionation. Typical gradient profiles are shown in Figure 4. In this

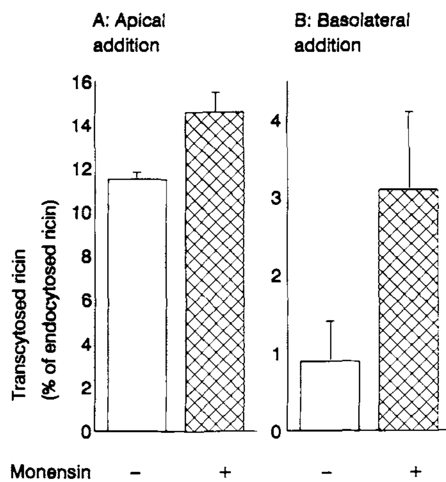

Fig. 2. Effect of monensin on transcytosis of ricin. Filter-grown cells were incubated for 15 min at 37°C with or without monensin (10 μM). ^{125}I-labeled ricin, (84–221 ng/ml; 23,600–36,850 cpm/ng), was then added, either to the apical surface or to the basolateral surface, and the cells were incubated for 1 h at 37°C. The amount of transcytosed ricin was measured as described in Materials and Methods. The data represent mean ± SD of 3 experiments.

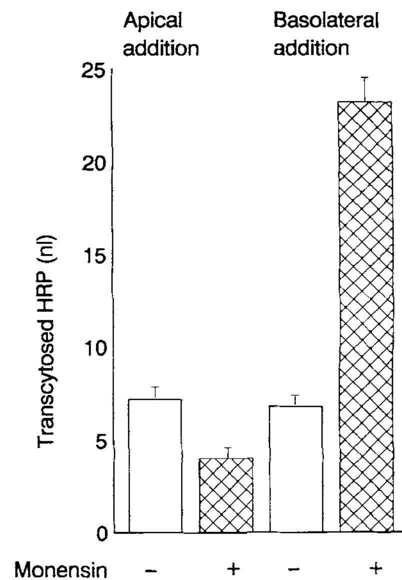

Fig. 3. Effect of monensin on transcytosis of HRP. Filter-grown cells were incubated for 15 min at 37°C in medium with or without monensin (10 μM). HRP (2 mg/ml) was then added, either to the apical side or to the basolateral side, and the incubation was continued for 1 h at 37°C. Finally, the cells were washed and the amounts of transcytosed HRP were determined as described in Materials and Methods. The amounts of HRP are expressed as volumes of fluid estimated as described in Materials and Methods. The data represent mean ± SD of 6 experiments.

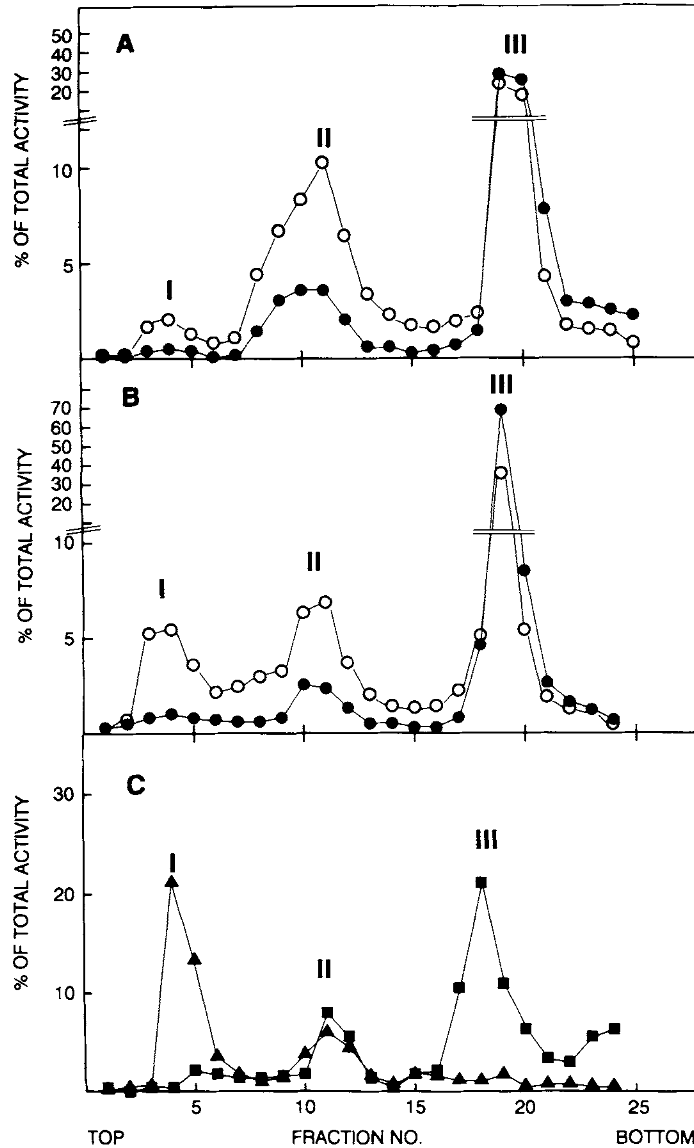

Fig. 4. Subcellular fractionation of MDCK cells incubated with ^{125}I-ricin and HRP. Cells growing on polycarbonate filters were incubated for 2 h at 37°C with and without 10 μM monensin and with ^{125}I-ricin (40,000 cpm/ng; 100 ng/ml) and HRP (4 mg/ml) added at the basolateral side. The incubation and subcellular fractionation were carried out as described in Materials and Methods. **Panel A** shows the distribution of ricin (○) and HRP (●) in the gradient after incubation without monensin; **Panel B** shows the corresponding distribution after incubation in the presence of monensin; **Panel C** shows the distrubution of UDP-galactosyl-transferase (▲) and hexosaminidase (■). Peak I represents Golgi-enriched fractions; peak II, the load zone; and peak III, endosomes and lysosomes.

gradient system the post nuclear supernatant (PNS) is loaded in the middle of the tube before centrifugation, and peak II represents the load zone. Endosomes and lysosomes (peak III) move downwards into the gradient (Sandvig et al., 1991a), and the Golgi apparatus (peak I) moves upwards (Sandvig et al., 1991a) to the 0.3 M/0.9 M sucrose interface. Figure 4A,B show the distribution of ^{125}I-ricin and HRP in gradients after incubation of cells without (A) and with monensin (B) and with basolateral addition of ricin and HRP. Figure 4C shows the distribution of the Golgi-marker UDP-galactosyltransferase and the lysosomal marker β-hexosaminidase.

Results from gradient fractionation experiments are summarized in Table IV. Monensin was found to increase the transport of ricin to the Golgi-enriched fractions both after apical and basolateral addition of toxin. The amounts of ricin in the Golgi fractions were highest at the

TABLE IV. Effect of Monensin on Endocytic Uptake and Transport of ^{125}I-labeled Ricin to the Golgi Complex*

Addition of toxin	Monensin concentration	Endocytosed ricin	Amount of ricin in the Golgi complex
	μM	% of control	% of endocytosed ricin
Apical	0	100	3.9 ± 0.3
	0.1	175 ± 5	4.0 ± 0.1
	10	215 ± 15	3.8 ± 0.1
Basolateral	0	100	5.2 ± 0.3
	0.1	93 ± 3	11.3 ± 0.5
	10	99 ± 6	14.2 ± 0.6

*Cells growing on polycarbonate filters were incubated with the indicated concentrations of monensin for 15 min at 37°C. Then ^{125}I-labeled ricin, (40,000 cpm/ng, 100 ng/ml), was added, either to the apical or to the basolateral surface together with 2–5 mg/ml HRP. The incubation was continued for 120 min at 37°C. The amount of endocytosed ricin and the amount of ricin in the Golgi complex was determined as described in Materials and Methods. The average and range between the values obtained in two experiments are shown.

highest concentrations of monensin tested. When ^{125}I-labeled ricin was internalized from the apical surface, the increase in the amount of Golgi-associated toxin was equal to the increase in endocytosed ricin (Table IV). As earlier found for shorter incubation periods (Table I), endocytosis of ricin from the basolateral side was not affected by monensin during the 2-h incubation in these experiments. The increased amount of toxin found in the Golgi apparatus upon addition of toxin basolaterally therefore seems to be due to increased transport of the endocytosed toxin to this organelle.

Cytotoxic Effect of Ricin Added at the Apical and Basolateral Sides

Since ricin transport to the trans-Golgi network appears to be a necessary step for intoxication of cells with this toxin, we tested the effect of monensin on ricin-mediated inhibition of protein synthesis when the toxin was added either at the apical side or at the basolateral side. The results showed that monensin (10 μM) sensitized the cells to ricin both when the toxin was added apically and basolaterally (Fig. 5). The cells were sensitized to about the same extent with 0.1 μM as with 10 μM of monensin (data not shown). This is in accordance with the data in Table IV that 0.1 μM monensin increased the transport of ricin to the Golgi complex to about the same extent as 10 μM.

DISCUSSION

The present results show that transcytosis of both HRP and ricin continues in filter-grown MDCK cells when the endosomal pH is increased by monensin. Therefore, low endosomal pH is not required for transcytosis as such, although it may be required for the transcellular transport of certain proteins (Mineo et al., 1990; Pesonen and Simons, 1983). In fact, the transcytosis of ricin was stimulated by monensin, both when the toxin was added basolaterally and apically. The stimulation of transcytosis of ricin from the apical surface is probably partly due to the increased accumulation of toxin inside the cells. However, transcytosis of ricin from the basolateral surface was increased without any concomitant increase in endocytosed toxin. It is possible that the increased pH as such can affect

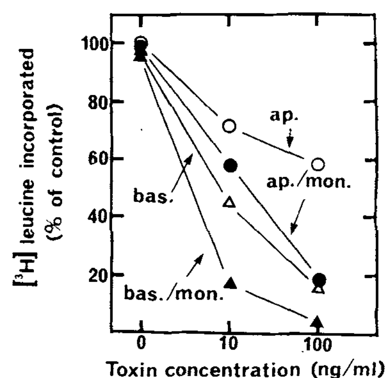

Fig. 5. Effect of monensin on the sensitivity of polarized MDCK cells to ricin. Cells growing on polycarbonate filters were incubated for 15 min at 37°C in Hepes medium with and without 10 μM monensin. Then, increasing concentrations of ricin were added to the apical or the basolateral side, and the cells were incubated for 3 h at 37°C before the protein synthesis was measured as described in Materials and Methods. (○), ricin added apically; (●), ricin added apically in the presence of monensin; (△), ricin added basolaterally; (▲) ricin added basolaterally in the presence of monensin.

the sorting, but it can not be excluded that structural changes of intracellular organelles caused by monensin (Sandvig et al., 1986) also may play a role.

In contrast to the results obtained with ricin, monensin reduced the transcytosis of the fluid phase marker HRP from the apical to the basolateral side. A possible explanation for this apparent discrepancy is that monensin reduces the size of the vesicles involved in transcytosis, thus decreasing the ratio of fluid phase to membrane that is transported across the cell layer. That this might occur on only one pole of the cell is not surprising in view of recent data demonstrating that the properties of early apical and basolateral endosomes are different (Bomsel et al., 1990). Also, the organization of microtubuli, which in the polarized MDCK cells are organized with most of the plus ends towards the basolateral pole and the minus ends directed towards the apical pole (Bacallao et al., 1989), could contribute to the different regulation of transport at the two poles. In fact, recently published findings show that only transcytosis from the basolateral to the apical pole is dependent on microtubuli (Breitfeld et al., 1990). Previous studies on HRP and ricin transport in filter-grown MDCK cells indicate differences between the two poles in the efficiency by which the internalized ligand is transcytosed (Bomsel et al., 1989; van Deurs et al., 1990), and, in the case of HRP, recycled (Bomsel et al., 1989). As shown here, also ricin recycling is most efficient at the apical pole. The observed reduction in recycling at the apical pole is probably the explanation for the increased accumulation of ricin, whereas in the case of HRP both reduced recycling and reduced transcytosis will contribute to the increased intracellular accumulation.

To quantify the amount of ricin transported to the Golgi apparatus, the method for fractionation of post-nuclear supernatants described by Sandberg et al. (1980) was modified and used to fractionate the MDCK cells. This modified method has been described previously (Sandvig et al., 1991a). The results showed that the Golgi-enriched fractions contained ricin, both when the marker had been added to the apical surface and to the basolateral surface. This is in agreement with results from ultrastructural studies which indicated that ricin was transported to TGN upon addition of toxin both at the apical and the basolateral pole (van Deurs et al., 1990).

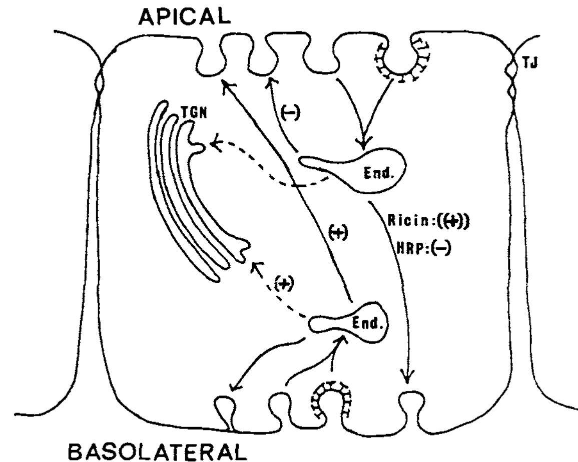

Fig. 6. Schematic illustration of the effect of monensin on intracellular pathways in polarized MDCK cells. (+), increased transport; ((+)), slightly increased transport; (−), decreased transport. End., endosomes; TGN, trans-Golgi network; TJ, tight junction. The broken lines between endosomes and the Golgi apparatus indicate that this pathway may involve transport via late endosomes.

Interestingly, monensin also increased the amount of ricin in the Golgi-enriched fractions. However, only after basolateral addition of ricin was there an increase in the fraction of endocytosed toxin transported to the Golgi apparatus. It is not clear whether or not this represents an increased transport from late endosomes to the Golgi apparatus, increased transport from early endosomes to late endosomes, or transport of ricin directly from early endosomes to the TGN. Since we cannot distinguish among these possibilities, we have used broken arrows between the endosomes and the Golgi apparatus in the schematic drawing in Figure 6.

Monensin not only increased the amount of ricin transported to the Golgi apparatus, but the ionophore also sensitized the cells to ricin. This is in agreement with earlier results (van Deurs et al., 1990; Sandvig and Olsnes, 1982; Sandvig et al., 1986) and is consistent with the idea that transport of ricin to TGN is a necessary step for intoxication of the cells (Sandvig et al., 1986, 1991b; Yoshida et al., 1990; van Deurs et al., 1989).

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