ELSEVIER

Biochimica et Biophysica Acta 1280 (1996) 27–33

BBA Biochimica et Biophysica Acta

Endocytosis inhibitors abolish the active transport of polypeptides in the mucosa of the nasal upper concha of the rabbit

Dario Cremaschi *, Cristina Porta, Roberta Ghirardelli, Claudio Manzoni, Italo Caremi

Dipartimento di Fisiologia e Biochimica Generali, Università degli Studi di Milano, Via Celoria 26, I-20133 Milano, Italy

Received 11 July 1995; revised 11 October 1995; accepted 1 November 1995

Abstract

An active absorption of polypeptides (elcatonin = CCT; adrenocorticotropic hormone) had been previously observed in the nasal respiratory mucosa of the rabbit. Its saturation kinetics and the parallel absence of a net transfer of other non-polypeptidic organic markers excluded the involvement of a simple pinocytosis. This absorption has been now better localized and further characterized. Unidirectional CCT fluxes (determined with radioimmunoassay) have been concomitantly monitored with transepithelial electric potential difference (V_{ms}). Although the mucosae covering the ectoturbinal A and the lower and upper conchae displayed similar V_{ms}, the active CCT transport was only evidenced in the upper concha. In this region cytochalasin B (which by disassembling actin microfilaments prevents the apical formation of vesicles in epithelial cells) and monensin (which prevents the split of the ligand-receptor complex in the endosomes) both eliminated the net CCT absorption, however, also permanently increasing the passive CCT junctional permeability. Aluminium fluoride (which prevents the fusion of endocytic vesicles into endosomes) and colchicine (which disrupts microtubules along which vesicles move in the cytoplasm) also permanently abolished net CCT transport, without affecting, or shortly and transiently affecting, passive permeability. On the whole these results are in favor of an active CCT transport supported by a specific vesicular transport.

Keywords: Calcitonin; Cytochalasin B; Colchicine; Monensin; Aluminium fluoride; Vesicular transport

1. Introduction

In a previous paper [1] we have evidenced an active net absorption of polypeptides (test molecules: elcatonin, M_w = 3362, and adrenocorticotropic hormone, M_w = 4500) across the respiratory mucosa of the upper part of rabbit nostrils, in a region approximately identified with the upper concha. The net absorption was considered active as it was abolished by chemical and physical metabolic inhibitors. Its saturation kinetics and the parallel absence of net transfer of other organic non-polypeptidic molecules, such as sucrose (M_w = 342) and polyethyleneglycol-4000 (M_w = 4000), indicated that the transport was not supported by a nonspecific basal pinocytosis. The probable physiological function was assumed hypothetically to be the sampling of potential antigens from nasal lumen to bring the information of their presence to underlying lymphoid tissues.

The aim of this paper is to better localize the nasal region where active absorption occurs and to check whether the transport is sensitive to known inhibitors of different functional steps involved in receptor-mediated endocytosis. For the latter purpose we have used: (i) cytochalasin B, which by disassembling actin microfilaments [2,3] prevents the apical formation of vesicles in epithelial cells [4], (ii) colchicine, which disrupts microtubules along which vesicles move in the cytoplasm [5,6], (iii) aluminium fluoride, which prevents the fusion of endocytic vesicles into endosomes [7], (iv) monensin, which prevents the reaching of a sufficiently low pH in the endosomes, thus blocking the split of the ligand-receptor complex [8,9].

2. Materials and methods

Male New Zealand rabbits (approximate weight: 3 kg) were killed by cervical dislocation; the fore-half nasal mucosae from the roofs of both nostrils were excised, washed with Krebs-Henseleit saline at room temperature

* Corresponding author. Fax: +39 2 70644702.

SSDI 0005-2736(95)00263-4

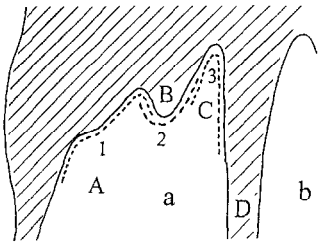

Fig. 1. Schematic drawing of transversal section of roof and walls of left nostril in the rabbit nose (nose fore-part, from the back). a, b, left and right nostrils; A, lower concha; B, ectoturbinal A; C, upper concha; D, septum. The dashed lines represent the areas of the mucosae used for the experiment and covering the lower concha (1), ectoturbinal A (2) and upper concha (3). The central part of these areas, for a width of 3 mm (length: 10 mm), was exposed between the two Teflon chambers for flux determinations.

and mounted between two Teflon chambers. The exposed area (0.3 cm^2) corresponded to that covering the lower nasal concha, the ecto-turbinal A or the upper nasal concha (Fig. 1). Histological preparations showed pseudostratified columnar ciliary epithelium (respiratory epithelium) to be largely predominant. The chambers were filled with 1 ml Krebs-Henseleit saline, bubbled with prehumidified 95% O_2 + 5% CO_2 to oxygenate the tissue, to maintain pH at 7.4 and to agitate the solution. Experiments were carried out at 27 ± 1°C as at this temperature the functionality of the isolated mucosa not only does not degrade but also improves with time for at least 150 min [10].

2.1. Measurement of transepithelial electrical potential difference (V_{ms})

Experiments were carried out at open electrical circuit. V_{ms} was measured by a digital multimeter (mod. 136, Keithley Instr., Cleveland, OH, USA); bridges were with 3% agar/3 M KCl. V_{ms} determinations were performed just after the tissue mounting to check the viability of the preparation: tissues with V_{ms} less than +1 mV (submucosa positive) were discarded. V_{ms} was then measured every 10 min for half an hour, before the start of flux determinations, and then at the end of the experiment (150 min from tissue mounting) both to check whether its time course was as previously observed [10] (controls) and to examine the effects of the inhibitors (when used) on this basic electrophysiological parameter.

2.2. Flux measurements

The only test molecule used was elcatonin ([1, 7 Asu] eel calcitonin; carbocalcitonin, CCT; $M_w = 3362$). It was determined by radioimmunoassay (RIA), so as to measure only the intact or nearly intact molecule [1], with the aid of a γ-counter (Autogamma 800C, Packard Instr. Co., Meriden, CT, USA). Teflon chambers were used for flux measurements to avoid surface adsorption of the polypeptide molecules. Mucosa-submucosa flux (J_{ms}) and the opposite flux (J_{sm}) were determined one on the right, the other on the left mucosa from the same animal (with random combinations), the functional symmetry of the two mucosae having been demonstrated in advance [1,10]. After a 30 min preincubation to allow the tissue to recover after isolation, 1 ml of saline was introduced, with the test molecule, into the mucosal or submucosal chamber (donor saline) and the appropriate transepithelial flux subsequently determined by complete withdrawal and immediate replacement of the saline (1 ml volume). J_{ms} and J_{sm} were each measured every 30 min for 120 min. Both fluxes were considered unidirectional since the maximal concentration reached in the flux chamber was 10^{-3}–10^{-5} compared to that applied in the opposite chamber. Samples (25 μl) of the donor saline were taken every 30 min to monitor possible concentration decreases due to flux or metabolism. Actually elcatonin concentration slightly decreased with time (see also [1]): hence, the measured fluxes were corrected and referred each time by a proportion to the initial nominal concentration of the hormone (10 or 20 μg/ml) [1].

2.3. Bathing saline and inhibitors

The Krebs-Henseleit solution had the following composition (mM): 142.9 Na^+, 5.9 K^+, 2.5 Ca^{2+}, 1.2 Mg^{2+}, 127.7 Cl^-, 24.9 HCO_3^-, 1.2 SO_4^{2-}, 5.5 glucose. Cytochalasin B and monensin were previously dissolved in ethanol and with it added to the Krebs-Henseleit saline (2.5 μl ethanol/ml) to reach a final concentration of 10 μg cytochalasin B/ml (21 μM) and 25 μM monensin. Ethanol effects at this concentration were checked on V_{ms} in 5 experiments for 150 min: V_{ms} values and time course were statistically unchanged compared to controls (data not presented). Aluminium fluoride (AlF_4^-) was dissolved in the saline as 100 μM $Al_2(SO_4)_3$ + 4.7 mM KF, F^- being substituted iso-osmotically for Cl^-. Colchicine was directly solubilized in the saline to reach a final concentration of 20 μM. All inhibitors were added on both sides at the beginning of the experiment just after the first V_{ms} measurement and remained in the media for the entire experiment (i.e., during the 30 min preincubation and the 120 min incubation periods).

2.4. Materials

Elcatonin and its antibody for RIA were supplied from ISF Laboratories for Biomedical Research (Trezzano sul Naviglio, Milan, Italy). Elcatonin labeling was carried out by Calbiochem Nova Biochem (Nottingham, UK). The RIA for elcatonin was previously set up in the ISF laboratories [11]. Cytochalasin B, $Al_2(SO_4)_3$, KF, colchicine and monensin were supplied by Sigma (St. Louis, MO).

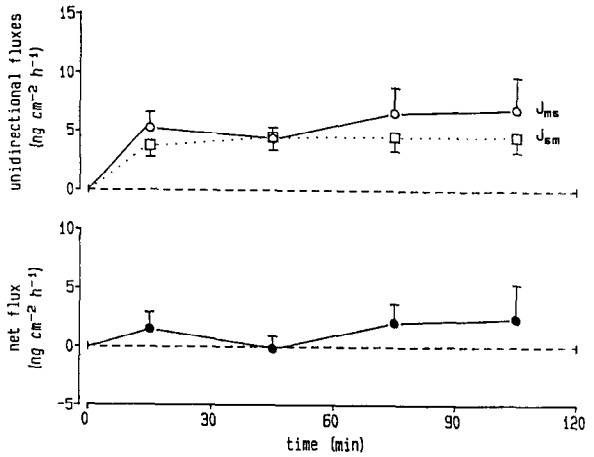

Fig. 2. Lower concha. Time course of unidirectional and net fluxes of elcatonin (10 μg/ml) under control conditions. Results presented as means ± S.E. ($n = 12$).

2.5. Statistics

Results are expressed as means ± standard errors; one-way ANOVA test was generally used for statistical analysis. In one case (as is indicated) paired data t-test was applied.

3. Results

3.1. Elcatonin absorption in different regions of the nasal mucosa

The mucosa covering the lower concha (Fig. 1) did not display any significant net absorption of elcatonin (CCT) in any experimental period (Fig. 2). Actually, CCT J_{ms} (for a CCT concentration of 10 μg/ml) statistically overlapped J_{sm} which was only slightly greater than that previously observed (Fig. 2 and Ref. [1]).

In the region of mucosa covering the ectoturbinal A (Fig. 1) the CCT net absorption was small (about 6 ng cm^{-2} h^{-1}) but statistically not significant at any time, without any peak at the 1st period (Fig. 3). Actually, J_{ms}(CCT concentration: 20 μg/ml) was not significantly different from J_{sm} at any period and its time course was similar to that of J_{sm} (Fig. 3). Both unidirectional fluxes were significantly much larger than those previously measured with the same CCT concentration (e.g., in the first 30 min period J_{sm} was 6–7-times higher: Fig. 3 and Ref. [1]). Thus, CCT passive permeability in this region seems to be relatively large.

The region of mucosa covering the upper concha (Fig. 1) should better correspond to that previously studied [1]. In fact it exhibited a net absorption significantly different from zero for 60 min and marginally significant ($P < 0.07$) in the subsequent period, with a time course similar to that previously measured and in the 1st 30 min period only a slightly larger peak (about 15 vs. 11 ng cm^{-2} h^{-1}: Fig. 4). Accordingly, CCT J_{ms} (CCT concentration: 20 μg/ml) was significantly higher than J_{sm} for 60 min (and

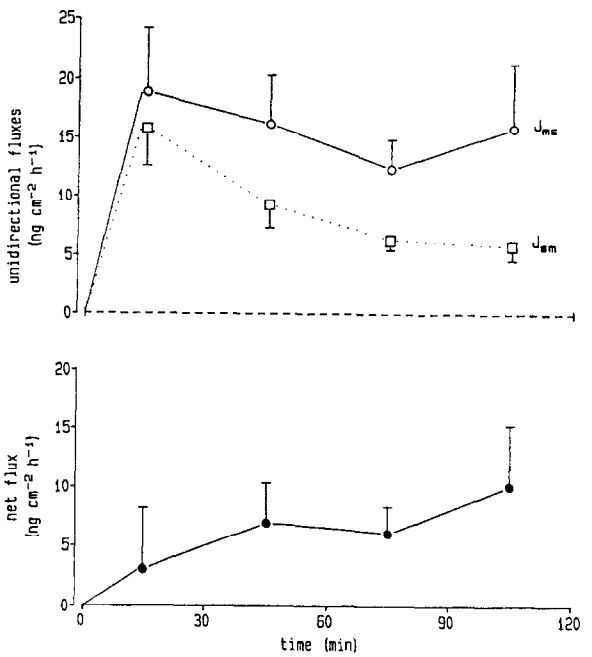

Fig. 3. Ectoturbinal A. Time course of unidirectional and net fluxes of elcatonin (20 μg/ml) under control conditions. Results presented as means ± S.E. ($n = 6$).

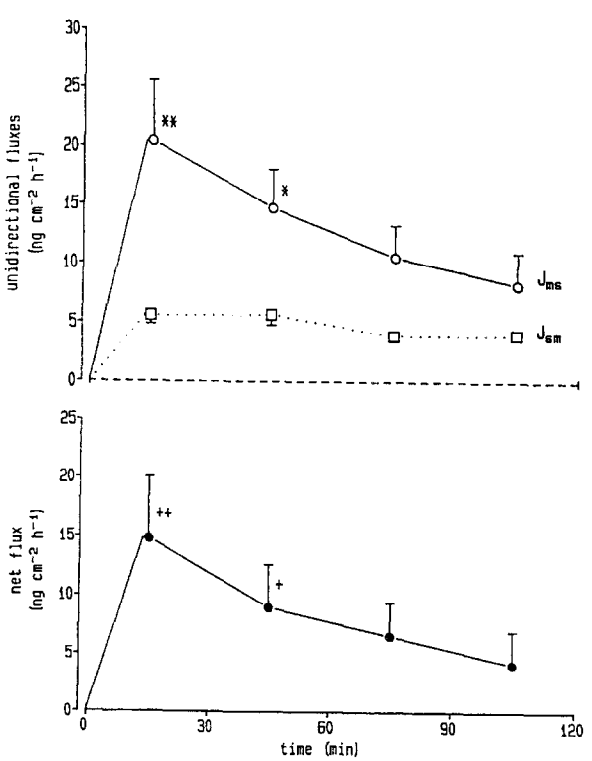

Fig. 4. Upper concha. Time course of unidirectional and net fluxes of elcatonin (20 μg/ml) under control conditions. Results presented as means ± S.E. ($n = 22$). *, **: $P < 0.05$ or 0.01 (compared to J_{sm}). +, ++: $P < 0.05$ or 0.01 (compared to zero).

marginally in the subsequent period, $P < 0.07$), with a maximum already reached in the 1st 30 min and a progressive decrease during the subsequent periods (Fig. 4). The time course was very similar to that previously observed, although the peak was larger by about 7 ng cm^{-2} h^{-1} [1]. J_{sm} was about 2-times greater than that previously measured with the same CCT concentration; however, it displayed the same time course, reaching its maximal value already in the 1st 30 min period and thereafter remaining at a plateau.

3.2. General electrical properties of different regions of the nasal mucosa

In spite of the differences in polypeptide absorption and passive CCT permeability observed in the three regions studied, V_{ms} displayed values and time courses similar in all the three regions (Fig. 5) and to those previously

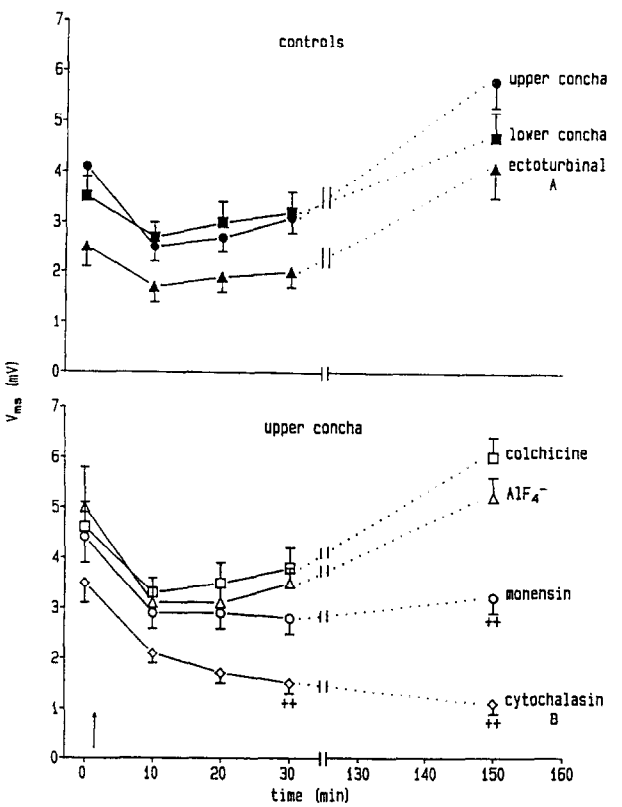

Fig. 5. V_{ms} time course in different mucosa regions (controls) and under different treatment conditions (region: upper concha). V_{ms} values were measured across the mucosae on which CCT fluxes were determined during the incubation period (30–150 min). Results presented as means + S.E. Number of experiments: for the controls on upper and lower conchae and ectoturbinal A $n = 44$, 24, 12, respectively; for tissues treated with colchicine (21 μM), AlF_4^- (100 μM), monensin (25 μM), cytochalasin B (10 μg/ml) $n = 28$, 22, 20, 22, respectively. The arrow shows the addition of the inhibitor. + +: $P < 0.01$ compared to the corresponding value measured under control conditions in the mucosa of the upper concha. All values measured at 150 min (with the exception of the values measured under monensin or cytochalasin B treatment) were significantly different ($P < 0.01$) compared to the corresponding values determined at 10 min.

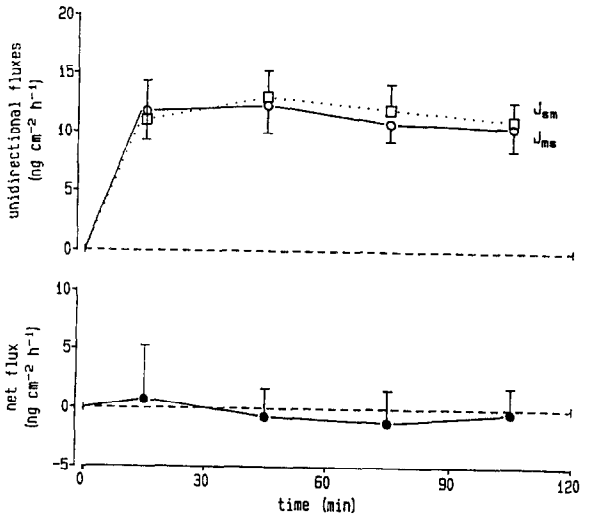

Fig. 6. Upper concha. Time course of unidirectional and net fluxes of elcatonin (20 μg/ml) under treatment with cythocalasin B (10 μg/ml). Results presented as means ± S.E. ($n = 11$).

measured [1,10], with a decrease in the first 10 min after isolation and a slow increase thereafter for at least 150 min ($P < 0.01$). For all the three regions the V_{ms}, measured in the paired mucosae (left and right) used to determine CCT J_{ms} and J_{sm}, were not statistically different from each other (paired data t-test) and were pooled together in Fig. 5. On this basis, mucosae on which elcatonin J_{ms} and J_{sm} were determined appeared to be symmetrical, as previously observed [1,10].

3.3. Upper concha. Cytochalasin B effects

When the tissue taken from the upper concha was treated with 10 μg cytochalasin B/ml (see Section 2), V_{ms} progressively decreased so that, at the end of the 150 min observation, it was reduced to about 30% (Fig. 5). Hence, this inhibitor seems to affect ion permeability and/or sodium pumping and/or metabolism. Elcatonin J_{sm} rose rapidly in the first 30 min of the incubation period to 11 ng cm^{-2} h^{-1} and remained stable during the subsequent periods (Fig. 6). Its values were about 2–3 times greater than the ones observed under control conditions ($P < 0.01$), which indicates a permanent increase in junctional permeability. J_{ms} values and time course were not statistically different from those of J_{sm} so that J_{net} proved to be abolished at any time (Fig. 6).

3.4. Upper concha. Colchicine effects

Experiments have also been performed by treating the tissue with 20 μM colchicine (see Section 2). V_{ms} values and time course were not significantly modified by the inhibitor (Fig. 5); thus, it seems that metabolism, Na^+ pumping, cell and junction ion permeabilities were not affected. Elcatonin J_{sm} increased transiently to 12 ng

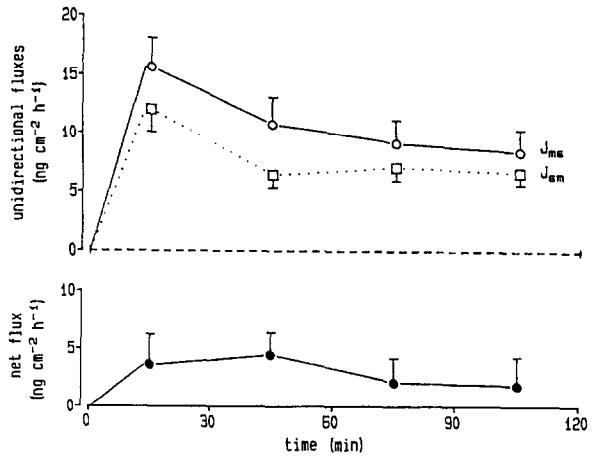

Fig. 7. Upper concha. Time course of unidirectional and net fluxes of elcatonin (20 μg/ml) under treatment with 21 μM colchicine. Results presented as means ± S.E. ($n = 14$).

cm^{-2} h^{-1} in the first 30 min period but then decreased and settled at values only slightly larger than those measured under control conditions (Fig. 7). J_{ms} was slightly greater than J_{sm} at each point-value; however, the small flux difference ($J_{net} = 2–4$ ng cm^{-2} h^{-1}) was only marginally significant at the second 30 min period (Fig. 7). In conclusion, colchicine largely reduces and even abolishes elcatonin net absorption, only shortly and transiently modifying the passive junctional permeability to the polypeptide.

3.5. Upper concha. Aluminium fluoride effects

By treating the mucosa of the upper concha with 100 μM AlF_4^- (see Section 2) V_{ms} values and time course proved to be unchanged compared to controls (Fig. 5) so that, as well as for colchicine, metabolism, Na^+ pumping, cell and junction ion permeabilities seemed to be unaffected by treatment. Elcatonin J_{sm} values and time course were statistically undistinguishable from controls (Fig. 8). Conversely, J_{ms} was strongly depressed and statistically

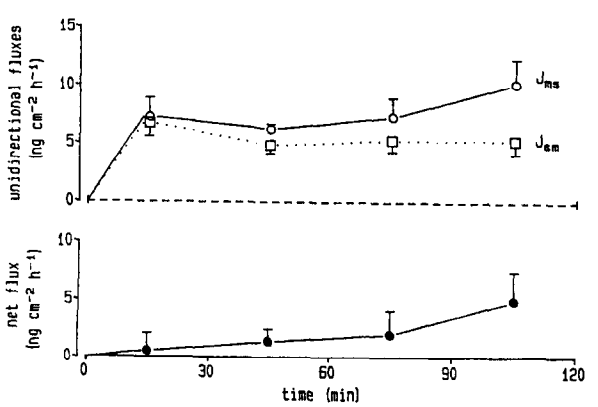

Fig. 8. Upper concha. Time course of unidirectional and net fluxes of elcatonin (20 μg/ml) under treatment with 100 μM AlF_4^-. Results presented as means + S.E. ($n = 11$).

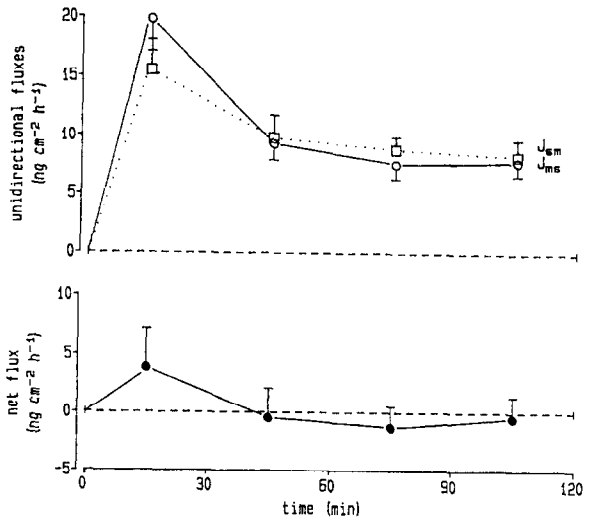

Fig. 9. Upper concha. Time course of unidirectional and net fluxes of elcatonin (20 μg/ml) under treatment with 25 μM monensin. Results presented as means ± S.E. ($n = 10$).

overlapped J_{sm}; as a consequence, J_{net} proved to be abolished at any time.

3.6. Upper concha. Monensin effects

In mucosae, excised from upper conchae and treated with 25 μM monensin (see Section 2), during the preincubation period V_{ms} displayed values and time course similar to those observed under control conditions; however, during the incubation period, it exhibited a significantly lower increase ($P < 0.01$) (Fig. 5). Thus, active and/or passive ion transport seemed to be moderately but significantly affected by treatment. In parallel CCT J_{sm} transiently increased to about 15 ng cm^{-2} h^{-1}, then decreased and stabilized at about 8.5 ng cm^{-2} h^{-1} (Fig. 9), a value about two times greater ($P < 0.01$) than that observed under control conditions. Thus, it seems that with this treatment the junctional passive permeability is permanently, although not drastically, modified. J_{ms} statistically overlapped J_{sm} at any time so that J_{net} proved to be abolished (Fig. 9).

4. Discussion

On the basis of the results obtained, the CCT active absorption clearly occurs across the epithelial covering of the nasal upper concha; conversely, it is negligible in the near region of the ectoturbinal A and nil in the lower concha. These differences in CCT net transport occur although general electrical properties appear to be similar in the three regions. CCT passive permeability in the ectoturbinal A region is higher than in the two conchae, but a few large junctional pores, increasing the per se very low basic CCT permeability without affecting significantly the V_{ms}, can explain this observation.

In the upper concha the net transport shows a maximum in the first 30 min, followed by a progressive decrease in the subsequent 90 min. This time course, in complete agreement with what we previously observed [1], is similar to that reported for the transcytosis of colostral antibodies, occurring in many neonatal intestines [12], and of uncoated or protein-coated microparticles, occurring in M cells of Peyer's patches [13–15]. We previously suggested that the polypeptide active transport may occur to sample antigens from the nasal lumen to the lymphoid tissue underlying the epithelium [1], as it happens in the intestinal Peyer's patches [16] and at the bronchial branch points [17]. If this hypothesis is correct, the transport decrease, whatever its cause, might be physiologically important in order that information be transferred from environment to organism without massively spreading antigens into the latter.

The transcellular active transport of macromolecules is generally based on vesicular transport and can result from a fluid-phase non-specific endocytosis (pinocytosis, FPE) or a receptor-mediated endocytosis (RME) [18]. The transcellular active transport of elcatonin in the nasal mucosa displays a saturation kinetics and a specificity for polypeptides [1]. Hence it is likely to be due to a RME. This process involves many known steps whose possible inhibitors are well identified. Among them we have used cytochalasin B, colchicine, aluminium fluoride and monensin.

Cytochalasins permeate cell membranes and disassemble actin filaments [2,3], thus inhibiting phagocytosis [19] and, in epithelial cells, the apical endocytosis [4], processes both supported by an actin cytoskeleton. Conversely, they do not affect endocytosis either in non-polarized cells or basolaterally in epithelial cells [4], where the fodrin/spectrin/ankyrin cytoskeleton seems to be involved [20]. These conclusions seem to be generally valid where epithelial endocytosis occurs [4]. In particular the actin-myosin I motor, present in microvilli [21,22],is shown to be involved in the apical pinching off of the coated pits (RME) and the noncoated caveolae (FPE) so that both ligand and fluid-phase internalizations are abolished by disassembling acting filaments [4].

On this basis the complete abolishment of elcatonin J_{net} in nasal mucosa by cytochalasin B is in agreement with a vesicular transport. We have observed some side-effects: (i) increase in CCT passive permeability, (ii) decrease in V_{ms}. The increase in CCT passive permeability, which indicates an increase in junctional permeability, is in accordance with the reduction in junctional resistance observed with this concentration of cytochalasin B in other epithelia, in which it has been shown that tight-junction permeability is under cytoskeletal control [23]. The decrease in V_{ms} is completed during the first 30 min (preincubation period) and the increase in CCT passive permeability is already observed at the start of incubation; thus, the rearrangement in junctional resistance should occur during the preincubation and in principle this can explain both events. However, cytochalasin B, although widely used to study the cytoskeletal dependence of many cellular phenomena, is also able to inhibit monosaccharide facilitated transport [24]. Since nasal mucosa functioning in vitro is partially dependent on exogenous glucose [10], the V_{ms} decrease and even the disappearance of CCT net flux might be also related to an ATP depletion. Hence, we have also treated the tissue with cytochalasin D, which is specific for actin disassembling and without action on monosaccharide transport [2]. The results obtained, as for both V_{ms} and CCT transport, were not significantly different from those reported here, obtained with cytochalasin B (Cremaschi D., Porta C., Ghirardelli R., unpublished data).

When vesicles have been translocated from the apical plasma membrane through the actin rich terminal web to the region below, once here, they are pulled along microtubules [6,25]. In the case of RME, receptor domains within the endosome can be immobilized by attachment to microtubules so that, after dissociation of ligand from receptors, ligand-containing endosomes can be pulled along microtubules away from the receptor [6]. Hence our results, showing that microtubule disruption abolishes the CCT net flux, with only weak side effects (a short and transient increase in CCT passive permeability) and without significant modifications of active ion transport and ion permeability (indexed by V_{ms}) are in favor of a vesicular transport of CCT.

In RME, to process the hydrolysis of the ligand-receptor complex, vesicles formed apically in the cytoplasm must fuse into endosomes. The process is under the control of heterotrimeric and monomeric G-proteins [7,26,27]. GTPγS, which activates both types of GTPases, both activates and inhibits endosome fusion; moreover it cannot permeate plasma membranes so that it can be used only for in vitro cell-free assays of the fusion [28]. Conversely AlF_4^-, which only activates the trimeric class of GTPases [28], thus inhibiting endosome fusion [30], is relatively membrane permeable so that can be used in intact cells [29].

On this basis the only abolition of CCT net flux we obtained with AlF_4^- (without modifications in CCT permeability and V_{ms}) is in agreement with a block of vesicle fusion and, as a consequence, with the model of active transport of CCT based on RME.

The effect we observed with monensin was more complex. Monensin is a protonophore which, promoting monovalent cation exchange across membranes, also prevents the acidification of endosomes and consequently the dissociation of the receptor-ligand complex and receptor recycling to the cell surface, thereby blocking the transcytosis [8,9]. Accordingly, CCT net transport was abolished. However, in this case both transient and permanent effects on paracellular passive permeability have been observed, which probably also account for a moderate inhibitory action on the transepithelial potential difference. These side-effects are probably related to pH variations of the

cytosol consequent to the Na^+/H^+ exchange due to the protonophore.

In conclusion, among the four inhibitors used, cytochalasin B and monensin reduce V_{ms} and permanently increase CCT junctional permeability, concomitantly with the abolishment of CCT net transport. Conversely, aluminium fluoride and colchicine permanently abolish net transport without any side-effect on junctional permeability and V_{ms} (aluminium fluoride) or only transiently affecting CCT junctional permeability (colchicine). Hence, the abolishment of CCT net transport by the former two inhibitors in principle might be also ascribed, at least partially, to the increase in junctional permeability. However, the action of the latter two inhibitors is univocal; moreover, the results themselves obtained with cytochalasin B and monensin, although not clear-cut, are at least not in contrast with a vesicular transport. On the whole the four inhibitors display a concordant action which, in addition to the previous observations that transport kinetics is saturating and non-polypeptidic organic markers are not absorbed, suggests that the polypeptide active transport existent in this epithelium is supported by a specific vesicular transport.

References

[1] Cremaschi, D., Rossetti, C., Draghetti, M.T., Manzoni, C. and Aliverti, V. (1991) Pflügers Arch. 419, 425–432.
[2] Cooper, J.A. (1987) J. Cell Biol. 105, 1473–1478.
[3] Copeland, M. (1974) Cytologia 39, 709–727.
[4] Gottlieb, T.A., Ivanov, I.E., Adesnik, M. and Sabatini, D.D. (1993) J. Cell Biol. 120, 695–710.
[5] Borisy, G.G. and Taylor, E.W. (1967) J. Cell Biol. 34, 525–533.
[6] Goltz, J.S., Wolkoff, A.W., Novikoff, P.M., Stockert, R.J. and Satir, P. (1992) Proc. Natl. Acad. Sci. USA 89, 7026–7030.
[7] Mayorga, L.S., Diaz, R. and Stahl, P.D. (1989) Science 244, 1475–1477.
[8] Harford, J., Wolkoff, A.W., Ashwell, G. and Klausner, R.D. (1983) J. Cell Biol. 96, 1824–1828.
[9] Kaiser, J., Stockert, R.J. and Wolkoff, A.W. (1988) Exp. Cell Res. 174, 472–480.
[10] Cremaschi, D., Rossetti, C., Draghetti, M.T., Manzoni, C., Porta, C. and Aliverti, V. (1991) Comp. Biochem. Physiol. 99A, 361–364.
[11] Tagliaro, F., Manzoni, C., Dorizzi, R. and Cristofori, S. (1988) Abstr. 14th Int. Congr. Biochem., Prague, Vol. 4, p. 200.
[12] Weaver, L.T. and Walker, W.A. (1989) in Human gastrointestinal development (Lebenthal, E., ed.), pp. 731–748, Raven Press, New York.
[13] Jenkins, P.G., Howard, K.A., Blackhall, N.W., Thomas, N.W., Davis, S.S. and O'Hagan, D.T. (1994) Int. J. Pharm. 102, 261–266.
[14] Pappo, J., Ermak, T.H. and Steger, H.J. (1991) Immunology 73, 277–280.
[15] Smith, M.W., Thomas, N.W., Jenkins, P.G., Miller, N.G.A., Cremaschi, D. and Porta, C. (1994) Exp. Physiol. 80, 735–743.
[16] Owen, R.L. (1983) Mucosal immunoregulatory mechanisms, pp. 88–98, Karger, Basel.
[17] Tenner-Racz, K., Racz, P., Myrvik, Q.N., Ockers, J.R. and Geister, R. (1979) Lab. Invest. 41, 106–115.
[18] Pastan, I. and Willingham, M.C. (1985) Endocytosis, pp. 1–326, Plenum Press, New York.
[19] Silverstein, S.C., Greenberg, S., Di Virgilio, F. and Steinberg, T.H. (1989) in Fundamental Immunology (Paul, W.E., ed.), 2nd Edn., pp. 703–719, Raven Press, New York.
[20] Nelson, W.J., Shore, E.M., Wang, A.Z. and Hammerton, R.W. (1990) J. Cell Biol. 110, 349–359.
[21] Mooseker, M.S. and Coleman, T.R. (1989) J. Cell Biol. 108, 2395–2400.
[22] Zot, H.G., Doberstin, S.K. and Pollard, T.D. (1992) J. Cell Biol. 116, 367–376.
[23] Bentzel, C.J., Hainau, B., Ho, S., Hui, S.W., Edelman, A., Anagnostopoulos, T. and Benedetti, L. (1980) Am. J. Physiol. 239, C75–C89.
[24] Bauduin, H., Stock, C., Vincent, D. and Grenier, J.F. (1975) J. Cell Biol. 66, 165–181.
[25] Fath, K.R., Mamajiwalla, S.N. and Burgess, D.R. (1993) J. Cell Science Suppl. 17, 65–73.
[26] Colombo, M.I., Mayorga, L.S., Casey, P.J. and Stahl, P.D. (1992) Science 255, 1695–1697.
[27] Gorvel, J.P., Chavrier, P., Zerial, M. and Gruenberg, J. (1991) Cell 64, 915–925.
[28] Chabre, M. (1990) Trends Biochem. Sci. 15, 6–10.
[29] Inoue, Y., Fishman, P.H. and Rebois, V. (1990) J. Biol. Chem. 265, 10645–10651.
[30] Colombo, M.I., Lenhard, J., Mayorga, L., Beron, W., Hall, H. and Stahl, P.D. (1994) Mol. Membr. Biol. 11, 2, 93–100.