Basolateral Localization and Transcytosis of Gonadotropin and Thyrotropin Receptors Expressed in Madin-Darby Canine Kidney Cells*

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Isabelle Beau, Micheline Misrahi, Babette Gross, Brigitte Vannier, Hugues Loosfelt, Mai Thu Vu Hai, Christophe Pichon, and Edwin Milgrom‡

From the Unité de Recherches Hormones et Reproduction, INSERM, Unité 135, Hôpital de Bicêtre, 94275 Le Kremlin Bicêtre, France

The thyrotropin (TSH) and follicle-stimulating hormone (FSH) receptors are present mainly on the basolateral cell surface in the thyroid gland and in Sertoli cells, whereas in ovarian and in testicular cells, the luteinizing hormone (LH) receptors are distributed throughout the cell surface. When expressed in Madin-Darby canine kidney (MDCK) cells, all three receptors accumulated at the basolateral cell surface showing that they carry the corresponding targeting signals. The receptors were directly delivered to the basolateral surface of the MDCK cells. A minor fraction of the gonadotropin receptors but not of TSH receptors was secondarily targeted to the apical surface through transcytosis. The mechanisms of basolateral targeting and transcytosis were analyzed using the FSH receptor as a model. Both were insensitive to brefeldin A and pertussis toxin. Gs activation by AlF4− and cholera toxin provoked a marked enhancement of FSH receptor transcytosis. The population of Gs proteins involved in this mechanism was different from that involved in signal transduction since neither FSH nor forskolin mimicked the effects of AlF4− and cholera toxin. Gs activation provoked a similar effect on LH receptor distribution in MDCK cells, whereas it did not modify the compartmentalization of the TSH receptor. Hormone-specific transcytosis was observed in MDCK cells expressing the gonadotropin (FSH and LH) receptors and was increased after cholera toxin administration.

The gonadotropin (LH1 and FSH) and thyrotropin (TSH) receptors belong to the large family of G-protein-coupled receptors (1–4). They possess the distinctive seven transmembrane spanning domains. However, they form a specific subgroup characterized by the presence of a large extracellular domain constituted by the repetition of leucine-rich motif (5). This ectodomain is responsible for the high affinity hormone binding (6–9). Gonadotropin and TSH receptors are mainly coupled to Gs and thus activate adenylate cyclase. The same receptors are also able to activate phospholipase C at high hormone concentrations. Mutations of the receptors have been described leading either to their constitutive activation (10–12) or in contrast to a loss of receptor function (13–15).

Little is known about the cellular trafficking of G-protein-coupled receptors in general and of this subgroup of receptors in particular. Ultrastructural immunocytochemistry has been used to analyze the internalization mechanisms of some receptors (16–18). LH receptor-driven hormone transcytosis was observed through endothelial cells of testicular blood vessels (19). Recently immunocytochemistry using specific monoclonal antibodies demonstrated the basolateral distribution of the TSH receptor in thyroid cells (20) and of the FSH receptor in Sertoli cells (21), whereas the LH receptor was present all over the cell surface of thecal, granulosa, and luteal cells in the ovary and Leydig cells in the testes (22, 23).

The question was thus raised as to whether these differences in cellular distribution were due to differences in the structure of receptors or simply secondary to the fact that thyroid follicular cells and Sertoli cells are polarized, whereas the LH receptor-expressing cells are not polarized. Another question raised was whether the mechanisms of receptor basolateral delivery were cell-specific or whether the receptors contained specific signals that could direct them to this membrane compartment in any polarized cell. To answer these questions we have established MDCK cell lines that express the FSH, LH, and TSH receptors. Using these models we have analyzed the distribution of the receptors in polarized monolayers.

MATERIALS AND METHODS

Cell Culture—MDCK cells (type II) were seeded and grown on coverslips (Nunc) or filters (0.4-µm polycarbonate, tissue culture-treated, Transwell costar) as described previously (24, 25).

Antireceptor Antibodies—Mouse monoclonal antibodies FSHR323 (21), LHR38 (26), and T5-51 or T5-317 (20, 27) recognize an epitope in the extracellular domain of the FSH, LH, and TSH receptors, respectively. Antibody T3-365 has been raised against the intracellular domain of the TSH receptor (20). The rabbit polyclonal TSHR-19-389 antibody raised against the extracellular domain of the TSH receptor was also used in some experiments.

Establishment of MDCK Cell Lines Permanently Expressing FSH, LH, and TSH Receptors—MDCK cell lines were obtained and maintained as described (28, 29) after cotransfection with expression vectors encoding either hFSHR (pSGS-hFSHR) (21), hTSHR (pSGS-hTSHR) (30), or pLHR (pCMV-LHRA) (8) and with the plasmid pSV-Neo which confers resistance to the antibiotic G418 (31). These clones were screened for receptor expression by immunocytochemistry using monoclonal anti-receptor antibodies. Sodium butyrate (10 mM) (Sigma) was used to increase the expression of the transfected genes as described (32). At least two different clones were studied for each receptor.
Verification of the Polarized Phenotype of Transfected MDCK Cells—

The transepithelial resistance of the clones was ~300 ohms/cm². Indirect immunofluorescence studies were performed as described (33) using antibodies (a gift of A. Le Bivic) directed against a basolateral (BC11) or an apical (BB18) endogenous proteins of MDCK cells. Polarization of an endogenous glycoprotein complex (25) was verified as described previously (34).

Purification and Western Blot Analysis of the Gonadotropin and TSH Receptors Expressed in MDCK Cells—These procedures were performed as described previously (20, 21, 26, 30).

Metabolic Labeling and Immunopurification of the Human FSH Receptor—Expression in MDCK Cells—After pulse labeling of MDCK cells expressing the human FSH receptor was performed as described previously (21). The FSH receptor was immunopurified as described (21).

Immunoassay—Indirect immunofluorescence was performed on MDCK cells grown to confluence on coverslips (33). The cells were washed with PBS (phosphate-buffered saline, Dulbecco’s formulation with 0.1 mM CaCl₂ and 1 mM MgCl₂) and fixed for 15 min in 3% paraformaldehyde in PBS*. After washing, the aldehyde groups were quenched with 50 mM NH₄Cl in PBS* for 30 min. After 1 h saturation with PBS*, 1% BSA (albumin, fraction V, Boehringer Mannheim) cells were incubated for 2 h at room temperature in a humid chamber with the monoclonal antireceptor antibodies (FSHR323, LHR38, or T5-317) in the presence of an excess of unlabeled hormone. The internalized fraction of the labeled receptor-antibody complex were performed as described previously (21). The cells were then fixed in 3% paraformaldehyde for 15 min and further processed as described above.

cAMP Assay—cAMP assay was performed as described (21, 26, 35). Cell stimulation was achieved with 50 nM hCG (Organon), 50 nM FSH (DuPont NEN) (90 μCi/ml) (ERIADiagnostic Pasteur)), or 125I-bTSH (70 μCi/ml) (DuPont NEN) in the absence or in the presence of an excess of unlabeled ligand for 10 min at 37 °C. For pertussis toxin (Sigma) treatment, two conditions were followed: before and throughout the experiment (39) or 1 μg/ml toxin throughout the experiment (41). For cholera toxin (Sigma) treatment, two conditions were followed: before (3 h) and throughout the experiment (39). No immunofluorescence could be observed in the absence of such a treatment (33). No immunofluorescence could be observed in the absence of such a treatment (33).

RESULTS

MDCK Cell Lines Permanently Expressing TSH, FSH, and LH Receptors—MDCK cells were transfected with expression vectors encoding the receptors and a gene imparting resistance to neomycin. Among neomycin-resistant clones, those giving the strongest immunocytochemical reaction with anti-TSH, anti-FSH, and anti-LH receptor monoclonal antibodies were selected. It was necessary to verify that these cell lines have conserved their ability to become polarized when grown to confluence. Three methods were used. 1) The transepithelial resistance was measured in all clones expressing TSH, FSH, and LH receptors; it was ≥300 ohms/cm² as has previously been observed for MDCK cells (43, 44). 2) The monoclonal antibody BC11 recognizes an antigen that has been shown to be restricted to the basolateral compartment of MDCK cells (not shown). 3) Predominant apical secretion of the 80-kDa protein (gp80 marker) was observed in control and transfected MDCK cells (not shown) (25).

It was also necessary to verify that these cell lines were expressing functionally active receptors of normal structure. In all cases, the receptors were enriched by immunopurification and then analyzed by Western blotting using specific monoclonal antibodies. In cells expressing FSH receptors two species were detected (Fig. 1) of apparent molecular mass 87 and 75-kDa protein, whereas the 87-kDa receptor was unchanged. These observations thus suggested that the 87-kDa species resulted in the transformation of the 81-kDa species into the 87-kDa form. In all cases, the receptors were enriched by immunopurification and then analyzed by Western blotting using specific monoclonal antibodies. In cells expressing FSH receptors two species were detected (Fig. 1) of apparent molecular mass 87 and 81 kDa. Treatment with peptide AlF₄⁻ (Sigma) (43, 44) which re-
kDa mature FSH receptor. Similar observations have been previously made in human ovaries and transfected L cells (21). Western blot experiments were also performed for LH and TSH receptors expressed in MDCK cells. In the case of the LH receptor (Fig. 1) two species were also observed at ~89 and 68 kDa. Endoglycosidase digestions showed that the former corresponds to the mature receptor, whereas the latter corresponds to the previously described mannos-rich-precursor of the LH/CG receptor (8) (data not shown). In the case of the TSH receptor antibodies raised against the extracellular domain detected three protein species (Fig. 1): one at ~120, one at ~90, and one at ~60 kDa. Similar molecular species have been previously described for the TSH receptor expressed in L cells (30); the largest species corresponds to the mature uncleaved receptor, the 90-kDa species to the mannos-rich-precursor, whereas the 60-kDa protein corresponds to the α subunit of the cleaved mature receptor. Antibodies raised against the intracellular domain of the receptor detected the two larger precursor species and also the 35–45-kDa β subunit of the cleaved mature receptor (not shown). In human thyroid cells, only cleaved mature receptor is present (traces of the mannos-rich precursor may be detected, however) (20, 30). Thus, in transfected MDCK cells, as well as in transfected L cells, the TSH receptor undergoes incomplete maturation and incomplete cleavage.

**Basolateral Localization of a Major Fraction of FSH, TSH, and LH Receptors in MCDK Cells**—Cells grown on coverslips were incubated on their apical surface with antireceptor antibodies. As shown in Fig. 3, FSH, TSH, and LH receptors could not be immunostained in these conditions. However, when the cells were treated with EGTA, opening the tight junctions and thus allowing the antibodies to reach the basolateral surface, immunostaining of the three receptors in the corresponding cells could be observed. The labeling decorated the cellular contours. As stated above, correct polarization of the confluent monolayers was verified with antibody BC11 directed against a basolateral endogenous antigen (Fig. 3). Antibody BB18 recognizing an apical endogenous antigen of MDCK cells was also used as control. A strong staining was obtained with this antibody in MDCK cells in the absence of EGTA treatment (not shown). For cells transfected with each receptor, analysis of immunofluorescence by confocal microscopy in EGTA-treated cells was performed. Fig. 4 shows the LH receptor distribution. Serial XY horizontal optical sections were performed from the apical to the basal membrane of transfected MDCK cells. Antibody BB18 (Fig. 4C, a–d) strongly stained the sections corresponding to the apical surface, whereas antibody BC11 (Fig. 4A, a–d) and the monoclonal antireceptor antibody (Fig. 4B, a–d) stained sections corresponding to the basolateral surface. The same conclusions were derived from XZ vertical sections (Fig. 4, f).

Immunocytochemistry or immunofluorescence do not yield quantitative data. Such data can be obtained by metabolic labeling of the receptor followed by either domain-selective biotinylation of the receptor (45) or surface immunoprecipitation (36). Both techniques can be applied separately to the basolateral and apical compartments yielding quantitative data. Such data can be obtained by metabolic labeling of the receptor followed by either domain-selective biotinylation of the receptor (45) or surface immunoprecipitation (36). Both techniques can be applied separately to the basolateral and apical compartments yielding quantitative data.
MDCK cells were performed from the apical (a) XZ cells and the corresponding the cells. The XY through the cells at 90° to the LHR38 (monoclonal antibodies BC11 (der “Materials and Methods.” Primary antibodies corresponded to processed for indirect immunofluorescence microscopy as described under “Materials and Methods.”) The antibody LHR38 (B) was also used. XY horizontal focal sections (parallel through MDCK cells) were performed from the apical (a) to the basal level (d) of the cells. The lower panel shows an XY section (c) of a selected area of cells and the corresponding XZ sections (f) taken in 0.1-μm steps through the cells at 90° to the XY sections. The apical region of the cells is oriented top-most. Bar, 10 μm.

measurements of receptors present on each surface. However, gonadotropin and TSH receptors are extremely “sticky” molecules that tend to aggregate. The biotinylation method thus led to the nonspecific precipitation of a major fraction of the intracellular mannose-rich receptor precursor (not shown). This problem still existed but was markedly reduced with the surface immunoprecipitation method. The latter was thus used for all further experiments. MDCK cells expressing FSHR, TSHR, or LHR were pulse-labeled with radioactive amino acids for 1 h (see “Materials and Methods”). Antireceptor antibodies were added either to the basal or to the apical compartments during the 3-h chase period. The receptor-antibody complexes were purified and analyzed by gel electrophoresis (Fig. 5). For FSHR and LHR ~85% of the receptor molecules were localized on the basolateral surface. In the case of the TSHR (which has not yet undergone cleavage after 3 h of chase (30)), the totality of the receptor molecules was found on the basolateral surface, no radioactive receptor being detected on the apical surface.

It has been previously observed that the distribution of some proteins may not parallel the distribution observed when studying their biological activity (46). We thus incubated polarized MDCK cells expressing the various receptors with the respective hormones applied either to the apical or to the basolateral compartments. Measurement of cAMP accumulation (Fig. 6) confirmed that the majority of active LH and FSH receptor molecules were present on the basolateral surface, whereas only minimal cAMP production was observed when the hormone was applied to the apical compartment. In the case of MDCK cells expressing TSH receptor, hormone-induced cAMP accumulation could only be observed in the basolateral compartment.

Targeting Pathways of FSH, LH, and TSH Receptors to Basolateral and Apical Membranes—Various pathways have been described for the polarized distribution of membrane proteins in epithelial cells (reviewed in Refs. 47, 48). After their synthesis, the proteins may be directly delivered to the basolateral or the apical surface; they may be delivered to one of these surfaces and then may undergo a transcytosis to the opposite surface (49), or they may be randomly delivered to both surfaces but thereafter stabilized in one of the compartments by interaction with some specific component of this compartment (50). To distinguish between these various possibilities, we followed the kinetics of appearance of the newly synthesized FSH receptor on the basolateral and apical cell surfaces. This was performed by metabolic labeling of the cells followed by selective surface immunoprecipitation at various time intervals.

As shown in Fig. 7, FSH receptor was initially detected on the basolateral surface thus suggesting a direct sorting to that compartment. The apical receptor was observed only at later chase times. These results implied that transcytosis from the basolateral compartment may be responsible for the low amount of receptor present on the apical cell surface. To further test this hypothesis, we used surface trypsin digestion. Cells were incubated with trypsin either in the apical or basolateral compartments, and the result of this treatment on receptor delivery to the opposite compartment was examined. When trypsin was applied to the apical compartment, the apical receptor disappeared, but there was no change in the concentration of the basolateral receptor. On the contrary when trypsin was applied to the basolateral compartment both the basolat-
eral and the apical receptors disappeared (Fig. 8). These observations demonstrated a direct basolateral targeting of receptor followed by a limited transcytosis toward the apical surface.

Identical experiments were performed with MDCK cells expressing LH receptors with similar results (Fig. 8). In the case of TSH-expressing MDCK cells, addition of trypsin to the apical compartment did not change the concentration of basolateral receptor, confirming the direct basolateral targeting of the protein. However, apical immunoprecipitation in the absence of trypsin failed to detect any receptor molecules, and thus no transcytosis occurred (Fig. 8).

Effect of Brefeldin A and Effectors of Trimeric G Proteins on Receptor Polarization—The transport mechanisms of various proteins have been shown to be differentially affected by brefeldin A (37, 51, 52). It has been proposed that these differences were due to the existence of different populations of transport vesicles (53) or, alternatively, to the existence of several specific components which insert different proteins into specifically targeted vesicles (52). Brefeldin A could thus exert different effects on the sorting of specific proteins either by acting on a subset of the vesicles (54) or, alternatively, by inhibiting the insertion into a given subset of vesicles of only a fraction of proteins. It has also been proposed that trimeric G-proteins play an important role in cell surface compartmentalization, Gi being involved in apical transport whereas Gs was thought to be involved in basolateral targeting (41, 55, 56). To examine these mechanisms in the case of FSH receptors, the following experiments were performed.

MDCK cells expressing FSH receptor were incubated with increasing concentrations of brefeldin A (5–20 ng/ml) as described by Low et al. (37). It was verified that at all these concentrations, the drug did not open the tight junctions. Brefeldin A did decrease slightly the transepithelial resistance, as described previously (57), but the resistance was never lower than 150 ohms/cm². The concentration of FSH receptor at the basolateral and at the apical surface was unchanged by brefeldin A at various concentrations (see Fig. 9 for the effect of brefeldin A at 20 ng/ml).

Administration of pertussis toxin, which inactivates the Gα subunits, had no effect on receptor concentration at either the basolateral or the apical surface of the cells (Fig. 9). Thus, basolateral targeting of FSH receptor does not seem to be dependent on Gs.

We then tested the activity of aluminum fluoride (AlF₄⁻) which is known to activate the various heterotrimeric G-proteins. As shown in Fig. 9, this compound markedly increased the proportion of apical receptor whose concentration became equivalent to that of the basolateral receptor. Given the lack of modification of Gs, this result suggested a possible involvement of Gi in receptor targeting. We thus incubated the FSHR-
expressing cells with cholera toxin, which activates G\textsubscript{a}. This treatment also provoked a major modification of the distribution of the receptor, with a significant increase in the concentration of the apical receptor (Fig. 9). Such an effect of both AlF\textsubscript{4}\textsuperscript{-} and of cholera toxin could have been due to a reorientation of the initial targeting pathway of the receptor, the receptor being inserted in vesicles targeted to both apical and basolateral compartments. An alternative mechanism would involve an increased transcytosis of receptor after initial delivery to the basolateral surface. To distinguish between these two possibilities, we added trypsin to the basolateral compartment of cholera toxin-treated cells. If there was a direct targeting of the receptor to the apical compartment, the concentration of the apical receptor should not have been modified by trypsin treatment. On the contrary, in the case of increased transcytosis, the apical receptor should have disappeared since its proteolysis would have occurred during its residency on the basolateral cell surface. As shown in Fig. 9, the latter result was observed, clearly suggesting that G\textsubscript{a} activation leads to an enhanced transcytosis of the FSH receptor from the basolateral to the apical membrane.

Besides modifying the apical/basolateral ratio of receptor, both AlF\textsubscript{4}\textsuperscript{-} and cholera toxin increased the total concentration of receptor observed on the cell surface. A metabolic labeling experiment was performed on untreated cells and on cells treated by cholera toxin. This treatment did not change the rate of synthesis of the receptor (data not shown). Thus G\textsubscript{a} activation may modify the turn-over rate of the receptor, possibly by diverting some of the internalized receptor from the lyosomal degradation pathway (17) to the transcytotic pathway.

Since the FSH receptor is coupled to G\textsubscript{a} and as this coupling leads to activation of adenylate cyclase, it could be imagined that activation of the receptor by the hormone and the ensuing increase in cAMP concentration were responsible for receptor redistribution. To examine this hypothesis, MDCK cells expressing the FSH receptor were treated either with FSH or with forskolin (Fig. 9). These treatments did not change the pattern of polarization of the FSH receptor. There was no increase in the concentration of the apical receptor, as compared with that observed after incubation with cholera toxin or AlF\textsubscript{4}\textsuperscript{-}. These results suggested that the pool of G\textsubscript{a} molecules involved in receptor transcytosis is different from the pool of G\textsubscript{a} molecules which is coupled to the FSH receptor at the plasma membrane. They also implied that the effect of G\textsubscript{a} is direct or at least that it involves a downstream effector which is different from adenylate cyclase.

Given that FSH receptor polarization was modified by the activation of G\textsubscript{a}, we examined whether similar effects could be observed for LH and TSH receptors. To this end, MDCK cells expressing LH or TSH receptors were incubated with cholera toxin. The distribution of LH receptors (Fig. 10) was changed in a manner very similar to that observed for FSH receptors, with cholera toxin treatment provoking an important increase in apical receptor concentration. This increase was due to an increased transcytosis since trypsin digestion of basolateral proteins prevented enhancement of apical receptor concentration. Conversely, cholera toxin had no effect on TSH receptor polarization (Fig. 10).

**Hormone Transcytosis through MDCK Cells Expressing Gonadotropin and TSH Receptors**—We have observed a limited degree of receptor transcytosis from the basolateral to the apical compartments in FSHR- and LH-expressing MDCK cells. This transcytosis was markedly increased when G\textsubscript{a} was activated by cholera toxin. We thus wondered whether receptor transcytosis could be accompanied by hormone transcytosis.

**FIG. 10. G\textsubscript{a} activation and polarization of LH and TSH receptors in transfected MDCK cells.** MDCK cells expressing LH or TSH receptors were pulse-labeled with [35S]methionine and [35S]cysteine for 1 h. Cholera toxin was present during the pulse. They were then chased for 3 h with a medium containing unlabeled amino acids. Trypsin (50 μg/ml) was added (+) or not (−) to the basolateral compartment during this chase period. When trypsin was present in the basolateral compartment, soybean trypsin inhibitor (300 μg/ml) was added in the apical medium. Surface immunoprecipitation was performed with antibody LHR36 or with antibody TSHR19–389 (see “Materials and Methods”). The immunoprecipitates were analyzed by SDS-PAGE and fluorography. The sizes of the molecular mass markers are indicated on the left.

Cells were thus incubated on their basolateral surface with either 125I-FSH, 125I-hCG, or 125I-TSH, and hormone appearance on the apical side was monitored (Fig. 11). Only trichloroacetic acid-precipitable radioactivity was considered to be significant since 125I originating from proteolyzed, degraded hormone appears in part in the apical compartment. Furthermore, only saturable (suppressed by excess unlabeled hormone) receptor-mediated transcytosis was taken into account. A minimal level of transcytosis was observed in untreated FSH and LH receptor-expressing cells. This transcytosis was markedly increased by cholera toxin treatment. When control MDCK cells were used (LH receptor-expressing cells incubated with 125I-FSH and FSH receptor-expressing cells incubated with 125I-hCG), no hormone transcytosis could be detected. In the case of TSH receptor-expressing MDCK cells, no transcytosis could be observed either in the absence or in the presence of cholera toxin (Fig. 11).

**DISCUSSION**

Expression of gonadotropin and thyrotropin receptors in MDCK cells did not yield high amounts of mature proteins on the surface of the cells. Indeed the biosynthetic machinery of MDCK cells does not process these proteins very efficiently, and for all three receptors there is accumulation inside the cells of mannose-rich precursor glycoproteins (8, 21, 30). However the choice of MDCK cells for this study was justified by their efficient polarization and the large amount of information available about the cellular trafficking of various polarized surface proteins in these cells (24, 43, 58).

The physiological basolateral localization of FSH (21) and TSH (20) receptors in Sertoli cells and thyrocytes was reproduced in the heterologous MDCK cells. This suggested that the corresponding targeting signals are embodied in the proteins themselves and are functional in heterologous polarized cells. Furthermore, the basolateral localization of the LH receptor in MDCK cells, a receptor which is not physiologically polarized (22, 23), suggested that the LHR also possessed a basolateral targeting signal as the other members of this receptor subgroup. In vitro mutagenesis will allow us to identify the signals involved in all three receptors.

A basolateral localization has been previously observed for two other G-protein-coupled receptors, the α2A-adrenergic receptor in MDCK cells (38) and the parathyroid hormone recep-
Basolateral Targeting and Transcytosis of LHR, FSHR, and TSHR

A small proportion (10–15%) of the gonadotropin FSH and LH receptors are localized on the apical surface of MDCK cells (72). This phenomenon, which is very limited in MDCK cells, may be more important in other cell types. In endothelial cells of testicular vessels, receptor-mediated transcytosis of hormone has been described (19). This transcytosis allows the hormone to cross the endothelial barrier and to be concentrated at close contact of target cells. FSH has been observed in the rete testis fluid, perhaps resulting from a transcytosis through Sertoli cells (72).

The TSH receptor also displayed a basolateral localization in MDCK cells, but no transcytosis was observed, and there was no apparent effect of Gs activation. These differences between the gonadotropin and TSH receptor may be related to the existence of an interaction of the TSH receptor with a cellular or an extracellular component of MDCK cells. Interaction of Na+/K+-ATPase with the cytoskeleton has been shown to stabilize the protein at the basolateral surface (50). The possibility remains, however, that transcytosis may occur in other cell types (endothelial cells).

A small proportion (10–15%) of the gonadotropin FSH and LH receptors are localized on the apical surface of MDCK cells. In these cells, previous studies involving a number of proteins have shown that apical localization in most cases results from direct targeting (64). This does not seem to be the case for gonadotropin receptors that are delivered to the basolateral surface and secondarily undergo transcytosis to the apical surface.

Brefeldin A inhibits the basolateral localization of the low density lipoprotein receptor (65) and of the polymeric immunoglobulin receptor (52), whereas it is without effect on the polarization of various other proteins (37, 66) including the FSH receptor. Two hypotheses have been proposed to explain these differences: either different carrier vesicles may be used to reach the same compartment and brefeldin A might act by selectively blocking one of these pathways, or alternatively, the insertion of different proteins into a given set of vesicles is mediated by several mechanisms and brefeldin A may alter selectively only some of them.

Monomeric G-proteins, specially of the rab and ARF families, are involved in practically all of the steps of vesicular trafficking (67, 68). The role of the heterotrimeric G-proteins has only recently been studied. Several immunocytochemical studies revealed the presence of heterotrimeric G-proteins in various intracellular compartments (69). Stow et al. (70) initially showed a role for Go(30) in the secretion of proteoglycan by LLC-PK1 cells. Pimplikar and Simons (55) established that the apical and basolateral targeting of surface proteins was regulated in the trans-Golgi compartment by heterotrimeric G-proteins. These authors proposed a general mechanism whereby Gs inhibited basolateral transport whereas G(i) stimulated apical transport. G(i) has also been implicated in the transcytosis of the polymeric immunoglobulin receptor (41). These observations led to discussions of hypothetical receptors or sorter proteins coupled to these vesicular G-proteins and of possible regulatory mechanisms (69, 71). The G-proteins might regulate the formation of vesicles or might facilitate the incorporation of the cargo molecules in the vesicles by interacting with a coat protein (56). The FSH receptor does not follow the model proposed by Pimplikar and Simons (55) since inhibition of G(i) was without effect on receptor distribution, whereas stimulation of G(i) did not increase direct receptor apical transport but enhanced receptor transcytosis. This may be due to the rerouting of internalized receptor from the degradative lysosomal pathway toward the transcytotic pathway. Such a mechanism would explain the increased concentration of total surface receptor without change in receptor rate of synthesis.

The compartmentalization and surface targeting of the LH receptor closely resembled those of the FSH receptor in MDCK cells. For both receptors, a minimal transcytosis of receptor and hormone is observed which is increased by G(i) activation. This phenomenon, which is very limited in MDCK cells, may be more important in other cell types. In endothelial cells of testicular vessels, receptor-mediated transcytosis of hormone has been described (19). This transcytosis allows the hormone to cross the endothelial barrier and to be concentrated at close contact of target cells. FSH has been observed in the rete testis fluid, perhaps resulting from a transcytosis through Sertoli cells (72).

FIG. 11. Transcytosis of FSH (A), hCG (B), or TSH (C) in transfected MDCK cells. Polarized monolayers of transfected MDCK cells grown on filters (circles, MDCK FSHR, squares, MDCK LHR, triangles, MDCK TSHR) were incubated for 1 h in the presence (closed symbols) or in the absence (open symbols) of 10 μg/ml cholera toxin (see "Materials and Methods"). Cells were then incubated for 10 min with 125I-FSH (A), 125I-hCG (B), or 125I-bTSH (C) in the basolateral compartment. Parallel incubations were performed in the presence of 10 M NaCl or 10 μg/ml cholera toxin (see "Materials and Methods"). The compartmentalization and surface targeting of the LH receptor closely resembled those of the FSH receptor in MDCK cells. For both receptors, a minimal transcytosis of receptor and hormone is observed which is increased by Gs activation. This phenomenon, which is very limited in MDCK cells, may be more important in other cell types. In endothelial cells of testicular vessels, receptor-mediated transcytosis of hormone has been described (19). This transcytosis allows the hormone to cross the endothelial barrier and to be concentrated at close contact of target cells. FSH has been observed in the rete testis fluid, perhaps resulting from a transcytosis through Sertoli cells (72).

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It will be interesting now to analyze the mechanisms of receptor targeting in transfected endothelial cells. Furthermore, in vitro mutagenesis should allow the definition of the signals involved in receptor targeting, transcytosis, and endocytosis of this subgroup of G-protein-coupled receptors.

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Basolateral Targeting and Transcytosis of LHR, FSHR, and TSHR

5248