Transferrin stimulates iron absorption, exocytosis, and secretion in cultured intestinal cells

MARCO T. NUÑEZ AND VICTORIA TAPIA
Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile

Nuñez, Marco T., and Victoria Tapia. Transferrin stimulates iron absorption, exocytosis, and secretion in cultured intestinal cells. Am. J. Physiol. 276 (Cell Physiol. 45): C1085–C1090, 1999.—The cellular mechanism by which basolateral transferrin (Tf) produces an increase in apical-to-basolateral Fe flux in Caco-2 cells was analyzed. After a pulse of $^{59}$Fe from the apical medium, three types of basolateral $^{59}$Fe efflux were found: a $^{59}$Fe efflux that was independent of the presence of Tf in the basolateral medium, a $^{59}$Fe efflux in which $^{59}$Fe was left in the cell bound to Tf, and a Tf-dependent $^{59}$Fe efflux in which $^{59}$Fe came off the cell not bound to Tf. Furthermore, addition of Tf to the basolateral medium doubled the exocytosis rate of Tf and increased the secretion of apolipoprotein A, a basolateral secretion marker. Both apotransferrin and Fe-containing Tf produced similar increases in $^{59}$Fe efflux, Tf exocytosis, and apolipoprotein A secretion. The Ca$^{2+}$ channel inhibitor SKF-96365 inhibited both the Tf-mediated increase in transepithelial Fe transport and the secretion of apolipoprotein A. Thus the activation of transepithelial Fe transport by Tf seems to be mediated by Ca$^{2+}$ entry into the cells.

Iron is a trace element that is both essential and potentially toxic to cells (1, 25). For these reasons, intracellular and body Fe levels are tightly regulated. Body Fe homeostasis is achieved through the regulation of the amount of Fe absorbed in the intestine (4, 5, 7). Intestinal Fe absorption is traditionally divided into three sequential steps: the uptake of Fe from the intestinal lumen, an intracellular phase, and a transfer step, in which Fe passes from the cells to the blood plasma. The level of Fe in the intestinal epithelial cell regulates the uptake step (22). It is possible that the regulation of the uptake phase is due, in part, to the activity of divalent cation transporter 1 (DCT1), an Fe$^{2+}$-H$^{+}$ cotransporter, since the intracellular levels of Fe (10) regulate its expression. The transfer step is less well understood in terms of the molecular mechanisms that determine its functioning, yet computer simulation of in vivo mucosal Fe absorption kinetics suggests that this step is highly regulated (16).

Enterocytes have abundant transferrin (Tf) receptors in their basolateral membranes (3, 12, 18). Because polarized Caco-2 cells acquire considerable amounts of Fe through the basolateral endocytosis of Tf, we postulated that the basolateral endocytosis of Tf is part of the system by which intestinal cells sense body Fe levels (18). Moreover, the intestinal Tf receptors have considerable affinity for apotransferrin (apoTf) (18), and the internalization of apoTf results in a protracted endocytic cycle that sequesters the Tf receptor in intracellular compartments (17). Importantly, both apoTf and dieriffic Tf (dFeTf) were recently described as enhancers of Fe$^{2+}$ transport by Fe-deficient Caco-2 cells (2), so the endocytosis of Tf has still uncharted effects on the regulation of Fe absorption.

Unregulated intestinal Fe absorption occurs in hereditary hemochromatosis (reviewed in Ref. 11). A link between the Tf receptor and the regulation of Fe absorption was established from the observation that the protein coded by the hemochromatosis gene binds to the Tf receptor and decreases its affinity for Tf (6). It is therefore apparent that a better knowledge of the basolateral endocytosis of Tf by intestinal cells will foster our understanding of the regulation of Fe absorption in health and disease. Accordingly, this study was designed to gain knowledge of the cellular mechanisms by which basolateral Tf increases transepithelial Fe fluxes.

MATERIALS AND METHODS

Reagents. SKF-96365, Tf, polydonal rabbit anti-human Tf antibody, apolipoprotein A, and polydonal anti-human apolipoprotein A antibody were from Calbiochem (La Jolla, CA). Fetal bovine serum culture medium, deferoxamine, diethylenetriaminepentaacetate (DTPA), nitrilotriacetate (NTA), buffers, and salts were purchased from Sigma Chemical (St. Louis, MO). $^{59}$Fe and $^{55}$Fe, in the ferric chloride form, were from DuPont NEN (Boston, MA). $^{125}$I was from Comisión Chilena de Energía Nuclear (Santiago, Chile). Culture plasticware and Transwell bicameral inserts were from Costar (Cambridge, MA). To eliminate contaminant Fe, all buffer solutions were filtered through Chelex-100 (Sigma).

Cell culture. Caco-2 cells, from the American Type Culture Collection (HTB-37, Manassas, VA), were cultured in DMEM supplemented with 10% fetal bovine serum. Culture medium was changed every 2–3 days. For transport and binding experiments, cells were grown on 1-cm$^2$ polycarbonate cell culture inserts, with membranes of 0.4-µm pore size. Cells grown for 13–15 days were used (1).

Tf preparation and labeling with $^{59}$Fe or $^{125}$I. ApoTf, $^{59}$Fe-labeled Tf, and dFeTf were prepared from commercial Tf (Calbiochem) as described (18). For pulse-chase experiments, Tf was labeled with $^{125}$I using Iodo-Gen (Pierce Chemical, Rockford, IL) and used at most 1 wk from preparation.

Effect of Tf on transepithelial Fe transport. Insert-grown cells were incubated in Iscove medium with 10 µM $^{59}$Fe$^{3+}$, as the complex $^{59}$FeCl$_3$-NTA ($^{59}$Fe:NTA, 1:2.2 molar ratio) (21), added to the apical medium. The basolateral medium was Iscove medium with 2 µM apoTf, 2 µM dFeTf, or neither. The basolateral medium was also supplemented with 100 µM DTPA as a sink for transported $^{59}$Fe. Transepithelial Fe transport was determined by measuring the $^{59}$Fe radioactivity found in the basolateral medium as a function of time.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

0363-6143/99 $5.00 Copyright © 1999 the American Physiological Society C1085
Immunoprecipitation of externalized Tf. Caco-2 cells grown in 4.7-cm² inserts were pulsed for 30 min at 37°C with 10 µM ⁵⁹Fe-NTA in the apical medium and 0.5 µM ¹²⁵I-labeled apoTf or ³⁵Fe diFeTf in the basolateral medium. Surface-bound ¹²⁵I-Tf was eliminated with a mild acid wash, and the internalized ¹²⁵I-Tf was chased for 20 min into the basolateral medium consisting of saline supplemented with 2.5 µM rabbit monoferric Tf and 100 µM DTPA. This concentration of rabbit Tf was more than 10,000 times the concentration of human Tf in the chase medium. During the chase, the apical medium had 10 µM ⁵⁹Fe-NTA. After the chase, 20 µl of rabbit anti-human Tf immune plasma were added to the basolateral medium and the mixture was left to stand for 60 min on ice, followed by overnight incubation with 200 µl of 10% insoluble protein A (Sigma). The mixture was sedimented by centrifugation, and the pellet was washed three times with saline supplemented with 1 mM EDTA. The Fe saturation of Tf in the precipitates was determined from ¹²⁵I and ⁵⁹Fe radioactivity, and the pellet was washed three times with saline containing 1 mM EDTA and the precipitates was determined from ¹²⁵I and ⁵⁹Fe radioactivity. As a control, mock basolateral chase medium, containing rabbit monoferric Tf and ⁵⁹Fe, was subjected to the same procedure of immunoprecipitation. The ⁵⁹Fe radioactivity associated with this pellet was subtracted from the radioactivity of the chase samples.

Tf endocytosis and exocytosis. ¹²⁵I-Tf internalization—externalization studies were done in insert-grown Caco-2 cells as follows. Cells, in triplicate wells, were pulsed for 10 min at 37°C with 0.4 µM ¹²⁵I-apoTf in the basolateral medium. Surface-bound ¹²⁵I-Tf was removed with a mild acid wash (21), followed by two washes with saline, and the internalized ¹²⁵I-Tf was chased for 10 or 20 min at 37°C in the presence of 10 µM unlabeled apoTf in the basolateral medium. The apical medium contained 10 µM Fe-NTA through the whole process. After the chase, the cells were washed three times with saline containing 1 mM EDTA and the cell-containing filters were excised with a scalpel. The ¹²⁵I radioactivity in the cells, as well as that in the basolateral medium, was determined in a Packard Cobra 2 gamma-radioactivity counter. With this protocol, only the Tf that had ¹²⁵I label, whereas the bulk of the Tf in the chase medium was unlabeled and in the apoTf form. To determine the Fe content of the externalized Tf, the basolateral medium was concentrated in centriochrome concentrators (Amicon, Beverly, MA) and the Tf forms were separated by urea gel electrophoresis (15). The gels were stained with Coomassie blue and dried, and the banding of ¹²⁵I-Tf was determined by autoradiography. Additional quantification of the ¹²⁵I-Tf species was done by cutting the gels into 1.1-mm slices and measuring their ¹²⁵I radioactivity in a gamma counter.

Apolipoprotein A secretion. Insert-grown Caco-2 cells were preincubated in Iscove medium for 30 min at 37°C. The medium was then changed to either Iscove medium (control) or to Iscove medium plus 2 µM diFeTf or 2 µM apoTf. Aliquots of the basolateral medium were taken at 1, 2, 3, and 4 h. The aliquots were concentrated by 10% TCA precipitation and subjected to SDS-PAGE. Apolipoprotein A was determined by a Western blot assay using anti-human apolipoprotein A (Calbiochem) as primary antibody. The antigen-antibody complex was detected with the Renaissance Western blotting chemiluminescence kit (DuPont NEN). When the effect of SKF-96365 was tested, it was added to the basolateral medium at 5 µM final concentration.

Concurrent apical and basolateral Fe uptake. Insert-grown cells were incubated for various times at 37°C with 10 µM ⁵⁹Fe-NTA in the apical medium and 2 µM ³⁵Fe diFeTf in the basolateral medium. In this way, the Fe gained by the cells through apical uptake (³⁵Fe) and through the endocytosis of basolateral Tf (⁵⁹Fe) were independently evaluated. The ⁵⁹Fe cell-associated radioactivity was determined in a Packard liquid scintillation analyzer, while the basolateral ⁵⁹Fe radioactivity was determined in a Packard gamma-radioactivity counter.

Data analysis. Curve fitting was done using the GraphPad Prism program (GraphPad Software, San Diego, CA). In all experiments, variables were tested in triplicate wells, and the experiments were repeated two to six times.

RESULTS

Basolateral Tf increased the apical-to-basolateral ⁵⁹Fe flux. Recently, the stimulation of basolateral Fe release from Caco-2 cells by both apoTf and diFeTf was reported (2). There remained the possibility that the stimulatory effect reported was due to the driving of Fe transport equilibrium between the cells and the extracellular medium by Tf with unused Fe binding capacity, since loading of Tf with Fe-NTA results in a fraction of Tf that is not saturated with Fe (e.g., 94–96% saturation of Tf in Ref. 2). To exclude this possibility, in our experiments DTPA (100 µM) was present in the basolateral medium as a sink for transported ⁵⁹Fe, so that the differences observed can be unambiguously ascribed to an effect of Tf that is independent of its Fe-binding capacity. Confirming previous results (2), we found that basolateral apoTf increased the rate of apical-to-basolateral ⁵⁹Fe flux, an effect that was shared with diFeTf (Fig. 1A). A twofold increase was found in the present assay, whereas in the assay of Alvarez-Hernandez et al. (2) the increase was fourfold. The stimulation of ⁵⁹Fe flux was dose dependent for both apoTf and diFeTf (Fig. 1B). The stimulatory effect of apoTf and diFeTf was completely inhibited by SKF-96365, an inhibitor of receptor-stimulated Ca²⁺ entry channels (14, 26) (Fig. 1A). SKF-96365 inhibition was already maximal at 3 µM, and it was sustained up to 10 µM, the highest concentration tested (Fig. 1C).

Immunoprecipitation of externalized Tf. Caco-2 cells can both internalize apoTf (17) and efficiently remove Fe from the Tf-Fe complex during endocytosis (18). Therefore, both apoTf and diFeTf should be in the apo form during endocytosis. We tested the possibility that during recycling Tf could bind Fe in an intracellular compartment and bring it to the basolateral medium. To this end, insert-grown Caco-2 cells were pulsed with ¹²⁵I-apoTf or ¹²⁵I-diFeTf, and the internalized ¹²⁵I-Tf was then chased in a medium containing rabbit apoTf. During the whole process, the apical medium had ⁵⁹Fe-NTA. The chased ¹²⁵I-Tf was then precipitated from the basolateral medium with rabbit anti-human Tf antibody, and its Fe content was estimated from the ⁵⁹Fe radioactivity found in the pellet. After 20 min of chase, the ⁵⁹Fe saturation of externalized Tf was 23.1 ± 5.2 and 20.4 ± 2.3% for apoTf and diFeTf, respectively (means ± SD; n = 3), indicating that both Tf forms externalized with similar amounts of ⁵⁹Fe.
Pulse-chase of endocytosed apoTf and the Fe content of recycled Tf. Preliminary calculations indicated that the amount of Fe bound to externalized Tf was insufficient to account for the doubling in the Fe transport rate induced by Tf. To eliminate the possibility that the methodology used could be underestimating the amount of Tf-bound externalized Fe, a second experimental approach was used that allowed us to differentiate between Tf externalized from the cells and the bulk of the apoTf present in the chase medium (Fig. 2). Insert-grown cells were pulsed with 0.4 µM 125I-apoTf and then chased into a basolateral medium containing unlabeled apoTf. The 125I-Tf collected from the chase medium was resolved into its different Fe-containing species by urea-PAGE (Fig. 2A; protein staining). The distribution of 125I radioactivity was determined by autoradiography (Fig. 2B) and quantified by measuring the 125I radioactivity of gel slices (Fig. 2C). About 40% (range 32–45%; n = 4) of the externalized 125I-Tf migrated as a monoferric species, Tf with Fe in the NH₂-terminal position being the predominant form (Fig. 2B). The rest of the externalized 125I-Tf was apoTf. The amount of all the externalized species of Tf increased with time of chase (Fig. 2C). Because Coomassie blue staining of the basolateral medium did not show components other than the apoTf used in the chase (Fig. 2A), we concluded that only the 125I-Tf had some monoferric Tf. Thus two different experimental approaches demonstrated that most of the 59Fe acquired from the apical medium externalized to the basolateral medium not bound to Tf.

Concurrent determination of apical and basolateral Fe fluxes. Because Caco-2 cells very effectively remove both Fe atoms from diFeTf (18) and because diFeTf externalized with newly bound Fe, we measured the Tf-mediated exchange of Fe between the cells and the basolateral medium. Coincubation of cells with 59Fe-NTA in the apical medium and [55Fe]diFeTf in the basolateral medium revealed that the cells gained 55Fe from Tf at a rate of 11.1 pmol/h, whereas the basolateral medium received 59Fe at a rate of 14.2 pmol/h in the presence of diFeTf and at a rate of 6.6 pmol/h in the absence of diFeTf in the basolateral medium. In all assays, basolateral medium was also supplemented with 100 µM diethylenetriaminepentaaceticate. Plotted is amount of 59Fe radioactivity in basolateral medium as a function of incubation time. B: Caco-2 cells were incubated in Iscove medium + 10 µM 59Fe-NTA and various concentrations of either apoTf or diFeTf in basolateral medium. Plotted is amount of 59Fe radioactivity found in basolateral medium after 1 h of incubation as a function of transferrin (Tf) concentration. C: dose-response curve of SKF-96365 inhibition. Caco-2 cells were incubated with 10 µM 59Fe-NTA in apical medium and either apoTf or diFeTf with various concentrations of SKF-96365 in basolateral medium. Plotted is amount of 59Fe radioactivity found in basolateral medium after 1 h of incubation as a function of SKF-96365 concentration.
mated a steady-state rate of Tf cycling of 5.5 pmol/h. Because externalized Tf is 20% saturated with Fe, (Fig. 2), the Tf-induced, Tf-bound efflux of 59Fe was calculated at 2.2 pmol/h. Because the overall Tf-induced Fe efflux was 7.6 pmol/h, it is apparent that the largest fraction of the Tf-induced 59Fe efflux (5.4 pmol/h) was not bound to Tf.

Basolateral Tf increased both the exocytosis of internalized Tf and the basolateral secretion of apolipoprotein A. The nature of the Tf-induced basolateral Fe efflux was explored in exocytosis and secretion experiments. To that end, the exocytosis rate of internalized 125I-Tf was determined in the presence or absence of apoTf or diFeTf in the basolateral medium (Fig. 3). Tf, either in its apo or Fe-containing forms, increased the initial rate of 125I-Tf internalization 1.7 ± 0.3-fold (n = 3). These data agree with evidence showing a Tf-mediated increase of Tf receptor recycling in K-562 cells (13), A431 cells (9), and Jurkat and L2C cells (20).

Next, we determined whether the effect of Tf was circumscribed to the stimulation of its own exocytosis or whether it produced a more general effect in secretion. For that, we measured the secretion of apolipoprotein A, a protein secreted to the basolateral medium by

---

### Table 1. Quantification of basolateral iron fluxes

<table>
<thead>
<tr>
<th>Process</th>
<th>Fe Flux (pmol h⁻¹ · cm⁻² insert⁻¹)</th>
<th>%Maximal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL-to-cell Tf-mediated ⁵⁹Fe uptake</td>
<td>11.1 ± 0.8</td>
<td>100</td>
</tr>
<tr>
<td>Cell-to-BL ⁵⁹Fe flux, with Tf in BL</td>
<td>14.2 ± 1.5</td>
<td>100</td>
</tr>
<tr>
<td>Cell-to-BL ⁵⁹Fe flux, without Tf in BL</td>
<td>6.6 ± 0.5</td>
<td>46.5</td>
</tr>
<tr>
<td>Calculated values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical-to-BL, Tf-dependent ⁵⁹Fe flux</td>
<td>7.6</td>
<td>53.5</td>
</tr>
<tr>
<td>Apical-to-BL, Tf-dependent, Tf-bound ⁵⁹Fe flux</td>
<td>2.2</td>
<td>15.5</td>
</tr>
<tr>
<td>Apical-to-BL, Tf-dependent, not Tf-bound ⁵⁹Fe flux</td>
<td>5.4</td>
<td>38.0</td>
</tr>
</tbody>
</table>

Measured values of Fe flux are means ± SD of 3 experiments. Cells grown in inserts were incubated for various times with 10 µM ⁵⁹Fe-nitrilotriacetate in apical medium, in absence or presence in the basolateral (BL) medium of 1 µM ⁵⁹Fe-labeled transferrin (Tf) with Fe in both sites. Cell-associated ⁵⁹Fe radioactivity and basolateral ⁵⁹Fe radioactivity were used to determine rates of different BL Fe fluxes. Fe fluxes are given in pmol Fe·h⁻¹ · cm⁻² insert⁻¹. *Calculated as difference of ⁵⁹Fe flux into basolateral medium in presence and absence of Tf. †Calculated considering a steady-state Tf flux of 5.5 pmol · insert⁻¹ · h⁻¹ and 20% ⁵⁹Fe saturation for externalized Tf. ‡Calculated as difference between Tf-dependent ⁵⁹Fe flux and Tf-bound ⁵⁹Fe flux.

---

Fig. 2. A minor fraction of internalized apoTf externalized with bound Fe. Caco-2 cells were incubated for 10 min at 37°C with 0.4 µM ¹²⁵I-labeled apoTf in basolateral medium. ¹²⁵I-apoTf was replaced with 10 µM unlabeled apoTf, and internalized Tf was chased for different times into basolateral medium. Aliquots of basolateral medium were subjected to urea-gel electrophoresis. A: Coomassie blue staining of basolateral medium separated by SDS-PAGE electrophoresis. Lane 1, 10-min chase at 37°C; lane 2, 20-min chase at 37°C; lane 3, 20-min chase at 4°C; lane 4, 10-min pulse at 4°C followed by 20-min chase at 37°C; lane 5, mixture of standards (Tf stds) for apoTf, Tf with Fe in COOH-terminal position (Fe₃Tf), Tf with Fe in NH₂-terminal position (Fe₁Tf), and diFeTf. B: autoradiography of gel shown in A. C: lanes 1, 2, and 3 of gel in B were cut into 1.1-mm fractions, and their ¹²⁵I radioactivity was determined in a gamma-radioactivity counter. Small amount of radioactivity found in lanes 3 and 4 (B) probably reflects some ¹²⁵I-apoTf that was carried over from pulse step. This radioactivity was subtracted from radioactivity corresponding to apoTf when saturation of externalized Tf was estimated. cpm, Counts/min.

Fig. 3. Basolateral Tf increased exocytosis rate of Tf. ¹²⁵I-diFeTf was allowed to internalize for 30 min at 37°C in insert-grown Caco-2 cells. After ¹²⁵I label was washed out, efflux of internalized ¹²⁵I-Tf into basolateral medium was determined at different times, in presence or absence in basolateral medium of 2 µM apoTf or 2 µM diFeTf. Control inserts contained culture medium without additions. Data, expressed as rate of ¹²⁵I-Tf exocytosis as function of time, were adjusted to a single-exponential decay function. Y-intercept determined initial rate of ¹²⁵I-Tf exocytosis. Shown is 1 of 3 similar experiments.
Caco-2 cells (19). Both diFeTf (Fig. 4A) and apoTf (Fig. 4B) induced a sustained increase in the secretion of apolipoprotein A by Caco-2 cells. The Tf-mediated increase in apolipoprotein A secretion was completely inhibited by the Ca\(^{2+}\) channel inhibitor SKF-96365 (Fig. 4C).

**Discussion**

The results shown in this work indicate that basolateral Tf, in either its apo or its diferric form, increased the transepithelial flux of Fe. Two facts allow us to eliminate the possibility that the increased Fe efflux was due to the driving of an Fe transport process by basolateral Tf. First, the Fe efflux experiments were done in the presence of DTPA, an effective Fe chelator. Therefore, any mass effect in driving the exit of Fe from the cells was already present under the basal incubation conditions. Second, diFeTf, with no Fe-binding capacity available, and apoTf produced similar increases in the rate of Fe transport. Hence, in its most general context, the stimulatory effect of Tf can be described as an increase in Fe efflux that is dependent on Tf regardless of its Fe status.

We next explored the possibility that the Tf-dependent increase in Fe efflux was due to the binding of Fe by Tf during its endocytic cycle. Pulse-chase experiments indicated that the endocytosis of either apoTf or diFeTf resulted in the externalization of Tf ~20% saturated with Fe. This Fe was acquired from a pool of freshly internalized Fe, since immunoprecipitation of externalized Tf contained \(^{59}\)Fe that had newly entered the cell from the apical medium. Concurrent determination of apical-to-basolateral and endocytosis-mediated Fe fluxes indicated that Tf-bound Fe efflux was only ~30% of the total Tf-dependent Fe efflux. Therefore, most of the Tf-dependent Fe efflux represented Fe not bound to Tf.

The nature of the cellular mechanism by which Tf stimulated Fe efflux was further explored in experiments that determined the rate of Tf exocytosis and apolipoprotein A secretion. It is generally accepted that Tf endocytosis is a constitutive process (24), but in several cell types Tf binding increases the recycling rate of its receptor (9, 13, 20). The presence of Tf in the basolateral medium increased the rate of Tf exocytosis in Caco-2 cells. To discern whether this increase was an event particular to Tf exocytosis or whether it was part of a more generalized response of the cells to basolateral Tf, we determined the effect of Tf on the secretion of apolipoprotein A, a basolateral secretion marker. The observation that Tf also increased the secretion of apolipoprotein A was an indication that Tf induces a process that is common to both exocytosis and secretion. Because regulated exocytosis is often mediated by a transient increase in cellular Ca\(^{2+}\) (8), we tested the effect of SKF-96365, an inhibitor of receptor-stimulated Ca\(^{2+}\) channels (14, 26). We found that SKF-96365 inhibited the effect of Tf, an intriguing observation since the Tf receptor is not known to be a signal transduction receptor. Nevertheless, it was recently reported that Tf binding increases intracellular Ca\(^{2+}\) levels in Jurkat and L2C cells, by a mechanism involving extracellular Ca\(^{2+}\) entry (20). Our observations that the Tf-mediated increase in transepithelial Fe transport and apolipoprotein A secretion was abrogated by SKF-96365 suggest that the entrance of extracellular Ca\(^{2+}\) via receptor-activated Ca\(^{2+}\) influx channels (14, 26) could mediate the effect of Tf. If this is so, a link between Fe and Ca\(^{2+}\) metabolism should be further investigated.

In summary, the data presented here indicate that in Caco-2 cells basolateral Tf, independently of its Fe content, enhances Fe efflux from the cells into the basal medium. It is probable that a considerable fraction of the Tf-induced increase in Fe efflux was due to the stimulation of secretion, since Tf also increased the exocytosis rate of Tf and the secretion of apolipoprotein A. The Ca\(^{2+}\) channel inhibitor SKF-96365 blocked the stimulatory effect of Tf on Fe efflux and apolipoprotein A.

---

**Fig. 4.** Basolateral Tf increased apolipoprotein A secretion. A: insert-grown Caco-2 cells were preincubated in Iscove medium for 30 min at 37°C. Basolateral medium was then changed to either Iscove medium (control; –), or Iscove medium + 2 µM diFeTf (+). Aliquots of basolateral medium were taken at 1, 2, 3, and 4 h. Aliquots were concentrated and subjected to SDS-PAGE and Western blotting for apolipoprotein A. Observed stimulation of apolipoprotein A secretion was common to both diFeTf and apoTf. B: in an experiment similar to A, effect of apoTf on stimulation of apolipoprotein A secretion was assayed. C: effect of Ca\(^{2+}\) channel inhibitor SKF-96365 on Tf-induced apolipoprotein A secretion was tested. Cells were incubated for 3 h at 37°C in Iscove medium (control) or Iscove medium supplemented with 2 µM of either apoTf or diFeTf, with or without 5 µM SKF-96365. Basolateral media were then concentrated and subjected to SDS-PAGE and Western blotting for apolipoprotein A.
A secretion, an indication that the stimulatory effect of Tf may be mediated by Ca²⁺ influx.

This work was supported by Fondo de Ciencia y Tecnología Grant 1970465 and by a Cátedra Presidencial en Ciencia to M. T. Nunez.

Address for reprint requests and other correspondence: M. T. Nunez, Dept. de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile.

Received 2 November 1998; accepted in final form 28 January 1999.

REFERENCES