Cell Swelling-induced Translocation of Rat Liver Na\(^+\)/Taurocholate Cotransport Polypeptide Is Mediated via the Phosphoinositide 3-Kinase Signaling Pathway*  

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Cell swelling stimulates phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) in hepatocytes, and the PI3K signaling pathway is involved in cAMP-mediated translocation of sinusoidal Na\(^+\)/taurocholate (TC) cotransporter (Ntcp) to the plasma membrane. We determined whether cell swelling also stimulates TC uptake and Ntcp translocation via the PI3K and/or MAPK signaling pathway. All studies were conducted in isolated rat hepatocytes. Hepatocyte swelling induced by hypotonic media resulted in: 1) time- and medium osmolarity-dependent increases in TC uptake, 2) an increase in the \(V_{\text{max}}\) of Na\(^+\)/TC cotransport, and 3) wortmannin-sensitive increases in TC uptake and plasma membrane Ntcp mass. Hepatocyte swelling also induced wortmannin-sensitive activation of PI3K, protein kinase B, and p70S6K. Rapamycin, an inhibitor of p70S6K, inhibited cell swelling-induced activation of p70S6K but failed to inhibit cell swelling-induced stimulation of TC uptake. Because PD98095, an inhibitor of MAPK, did not inhibit cell swelling-induced increases in TC uptake, it is unlikely that the effect of cell swelling on TC uptake is mediated via the MAPK signaling pathway. Taken together, these results indicate that 1) cell swelling stimulates TC uptake by translocating Ntcp to the plasma membrane, 2) this effect is mediated via the PI3K, but not MAPK, signaling pathway, and 3) protein kinase B, but not p70S6K, is a likely downstream effector of PI3K.

Conjugated bile acids, like taurocholate (TC), are efficiently transported across hepatocytes and the Na\(^+\)-dependent TC uptake at the sinusoidal membrane of rat livers is mediated primarily via Ntcp (1, 2). The rat liver Ntcp is a \(~51\text{kDa}\) serine/threonine phosphorylated glycoprotein (3) with seven transmembrane domains (4–6). Na\(^+\)/TC cotransport and Ntcp are up-regulated by hormones and down-regulated by cholestatic agents (7–11). We demonstrated that cAMP, acting via protein kinase A, rapidly increases transport maximum of Na\(^+\)/TC cotransport in hepatocytes (12) by translocating Ntcp to the plasma membrane (13). The ability of cAMP to translocate Ntcp is dependent on cAMP-mediated increases in cytosolic [Ca\(^{2+}\)] (3) and protein phosphatase 2A (14). Recently, we reported that cAMP activates protein kinase B in hepatocytes and wortmannin, an inhibitor of PI3K, inhibits the ability of cAMP to stimulate PKB and to translocate Ntcp (15). These observations suggested a role for the PI3K/PKB signaling pathway in cAMP-mediated stimulation of Na\(^+\)/TC cotransport. However, cAMP failed to stimulate PI3K when assayed using whole cell lysate (16), suggesting that cAMP may activate the PI3K/PKB signaling pathway at a site downstream of PI3K. If the PI3K/PKB signaling pathway is involved in the regulation of hepatic Na\(^+\)/TC cotransport, other stimuli known to activate PI3K and PKB should also stimulate the cotransport. This hypothesis was tested by studying the effect of cell swelling on hepatic TC uptake.

Hepatocytes undergo changes in cell volume in response to hormones, nutrient supply and oxidative stress (17). A volume increase in hepatocytes is associated with the activation of PI3K and MAPK (18, 19). Cell swelling induced by hypotonic media or amino acids has been shown to activate glycogen synthase and acetyl-CoA carboxylase in isolated hepatocytes, effects postulated to be mediated via the PI3K signaling pathway (19). Cell swelling-induced activation of PI3K has also been suggested to modulate cholangiocyte ATP release and chloride secretion (20), and PI3K plays a significant role in volume regulation in a rat hepatoma cell line (21). Cell swelling also stimulates biliary excretion of TC, and this effect appears to be mediated via the MAPK signaling pathway (18). However, the role of MAPK in TC uptake is unknown. In the present study, we determined whether cell swelling induced by hypotonic media also stimulates TC uptake and whether this effect is mediated via the PI3K and/or MAPK signaling pathway. Our results are consistent with the hypothesis that cell swelling stimulates Na\(^+\)/TC cotransport via the PI3K/PKB signaling pathway.

**EXPERIMENTAL PROCEDURES**

**Materials—**TC (sodium salt), PD98059, and rapamycin were purchased from Cal-Biochem (San Diego, CA). Wortmannin, aprotinin, leupeptin, and collagenase were obtained from Sigma. \(^{[24\text{C}]}\)Taurocholic acid (56 mMCl/mmol) and \((\text{Met oxy}-\text{H})\)Hinulin (80 Cl/mmol) were purchased from NEN Life Science Products. Anti-fusion protein antibodies to the cloned Ntcp were prepared as described previously (5, 6) and were generous gifts from the laboratories of Drs. Suchy and Meier. Male Wistar rats (200–300 g) obtained from Charles River Laboratories served as liver donors.

**Hepatocyte Preparation—**Hepatocytes were isolated from rat livers using a previously described collagenase perfusion method (22). Freshly prepared hepatocytes suspended (100 mg wet weight/ml) in a HEPES assay buffer (pH 7.4) containing 20 mM HEPES, 140 mM NaCl, 5 mM...
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KCl, 1 mM MgSO\(_4\), 1.0 mM CaCl\(_2\), 0.8 mM KH\(_2\)PO\(_4\), and 5 mM glucose were incubated for 30 min at 37 °C under air before initiating studies. Cell swelling was induced by incubating hepatocytes in a hypotonic medium (prepared by decreasing NaCl concentration of the HEPES assay buffer). We first determined the effect of cell swelling on TC uptake and then biotinylated followed by separation of biotinylated proteins and determination of Ntcp using immunoblot analysis as described previously (15).

Biotinylation of Cell Surface Proteins—Cell surface proteins were biotinylated using a previously described method (15). Briefly, hepatocytes (200 mg wet wt/ml) pretreated with various agents were washed twice in ice-cold phosphate buffered saline (pH 5.0) and then exposed to sulfo-NHS-LC-Biotin (0.5 mg/ml; Pierce) in phosphate buffered saline for 3 h at 4 °C followed by washing 3 times with excess phosphate buffered saline. Cell pellets were resuspended in lysis buffer (20 mM Tris, 150 mM NaCl, 1% Triton, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 1 mM \(\beta\)-glycerophosphate, 10 mM/ml aprotinin, 10 mM/ml leupeptin, 500 mM \(\alpha\) okadaic acid, and 1 mM orthovanadate, pH 7.5) and then incubated for 1 h at 4 °C. The cell lysates obtained following centrifugation at 16,500 \(\times g\) for 5 min were used to determine biotinylated and total Ntcp mass. To assay for biotinylated Ntcp, the lysates were incubated with streptavidin-agarose beads for 1 h. The beads were separated by centrifugation following incubation with cell swelling and then boiled in Laemmli sample buffer for 5 min, followed by centrifugation. The resulting supernatant was also analyzed for the presence of actin to determine whether cytosolic proteins were also biotinylated by our procedure. Although actin can easily be detected in the whole cell lysate, no actin was biotinylated (data not shown), indicating that cytoplasmic proteins were not biotinylated.

Immunoblot Analysis—Proteins (5–200 \(\mu\)g) from plasma membranes, whole cell lysate, and supernatant containing biotinylated proteins were subjected to 12% SDS-PAGE by the method of Laemmli (24) as described previously (13). Proteins were transferred electrophoretically from SDS gels to nitrocellulose membranes (Transblot transfer membrane, 0.45 μm; Bio-Rad) and probed with the phospho-PKB (Akt-Thr308) antibody (1:1,000 dilution) to detect the activated form of PKB. The blot was stripped and reprobed with PKB (Akt) antibody (1:1,000 dilution) to detect total PKB. For MAPK, whole cell lysates (50–150 μg of total protein) were subjected to 10% SDS-PAGE. Separated proteins were transferred electrophoretically from SDS gels to nitrocellulose membranes and probed with the phospho-p44/42 MAPK (Thr202/Tyr204) antibody (1:1,000 dilution) to detect the activated form of MAPK. The blot was stripped and reprobed with p44/42 MAPK antibody (1:1,000 dilution) to detect total MAPK. In addition, MAPK was also assayed in selected samples using an enzyme-linked immunosorbent assay (ELISA) method. Briefly, activated MAPK from whole cell lysates was immunoprecipitated with phospho-p44/42 MAPK (Thr202/Tyr204) monoclonal antibody, followed by incubation with MAPK substrate (Elk-1 fusion protein), 10% SDS-PAGE, and detection of the product (phospho-Elk-1) using phospho-Elk-1 (Ser383) polyclonal antibody. The immunoblots were developed with an enhanced chemiluminescence kit (Amerham Pharmacia Biotech), according to the manufacturer’s instructions. Both methods yielded similar results.

The activity of p70\(^{60k}\) in cell lysate was determined using a peptide substrate provided by Upstate Biotechnology, Inc. The assay was conducted in the presence of three protein kinase inhibitors (protein kinase A, protein kinase C, and calcmodulin kinase), and the activity of p70\(^{60k}\) was obtained after subtracting endogenous substrate phosphorylation as described previously (15).

Other Methods—The Lowry method was used to determine cell protein (27). The blots and autoradiograms were scanned in gray scale using Adobe Photoshop© (Adobe System Incorporated, San Jose, CA), and the relative band densities were quantitated using Sigma Gel® (Jandel Scientific Software, San Rafael, CA). All values are expressed as the means ± S.E. Paired t test was used to statistically analyze data with p < 0.05 considered significant.

RESULTS

Effect of Cell Swelling on TC Uptake and Ntcp Translocation—To determine whether cell swelling affects hepatic TC uptake, hepatocytes were incubated in a hypotonic medium, and TC uptake was determined at different times. Cell swelling induced by hypotonic media resulted in time-dependent increases in TC uptake compared with uptake in control hepatocytes incubated in isotonic media (Fig. 1). TC uptake increased significantly at 10 min with a 2-fold increase observed at 25 min and no significant increase at 5 min. Moreover, the increase in TC uptake at 15 min was not significantly different from the uptake at 25 min, indicating that the maximum effect had been attained by 25 min. Thus, further studies on TC uptake were conducted following 25 min of exposure to hypotonic media. The increase in TC uptake was inversely proportional to medium osmolarity (Fig. 2).

To determine whether the increases in TC uptake was due to Na\(^+\)-dependent TC uptake, effect of cell swelling was determined in the presence and absence of extracellular Na\(^+\), with choline replacing Na\(^+\) in the media. It may be noted that isolated hepatocytes undergo similar changes in cell volume when incubated in choline, instead of Na\(^+\), containing media (28). In the absence of extracellular Na\(^+\), cell swelling failed to increase TC uptake (Fig. 3), indicating that the increase in TC uptake is primarily due to increased Na\(^+\)-dependent TC uptake.
Hepatocytes were exposed to isotonic (300 mosmol) or hypotonic (160 mosmol) HEPES buffer for 5, 10, 15, and 25 min followed by determination of initial uptake rate of TC. Concentration-dependent uptake of TC showed that the increase in TC uptake by cell swelling was due to an increase in maximum uptake rate (Fig. 4) and that the affinity constant ($K_m$) was not significantly affected (18 ± 0.69 versus 26 ± 4.05, μM). These results indicate that cell swelling stimulates Na+/TC cotransport by increasing the maximal transport rate.

We then determined whether the increase in TC uptake was due to translocation of Ntcp to the plasma membrane. We used a cell surface biotinylation technique to determine plasma membrane Ntcp mass. Hepatocytes incubated in hypotonic media expressed more Ntcp in the plasma membrane without changes in total cell lysate Ntcp (see Fig. 6). These results indicate that cell swelling stimulates Ntcp translocation to the plasma membrane.

**Role of PI3 Kinase on TC Uptake and Ntcp Translocation**—To determine whether the stimulatory effect of cell swelling on TC uptake and Ntcp translocation was mediated via the PI3K signaling pathway, we studied the effect of PI3K inhibitors, wortmannin, and Ly294002. Preincubation of hepatocytes with 200 nM wortmannin did not affect TC uptake under the isotonic condition but abolished the increase under the hypotonic condition (Fig. 5). Wortmannin alone did not affect either plasma membrane or total cell lysate Ntcp mass (Fig. 6). When hepatocytes were exposed to hypotonic media, plasma membrane Ntcp mass increased by 1.8 ± 0.18-fold, and this effect was abolished by pretreatment with wortmannin (Fig. 6). Similar results were obtained with Ly294002 (data not shown). Thus, increases in TC uptake and Ntcp translocation induced by cell swelling are likely to be mediated via the PI3K signaling pathway.

**Effect of Cell Swelling on PI3K and PKB Activity**—Cell swelling induced by hypotonic media has previously been shown to activate PI3K in hepatocytes (19). To confirm this under our experimental conditions, we determined PI3K activity in hepatocytes exposed to hypotonic media (160 mosmol) for 25 min followed by determination of initial uptake rate of TC at various concentrations. Data were fitted to the Michaelis-Menton equation to determine $K_m$ and $V_{max}$ using a statistical program, and the solid lines represent the best fit lines. Data represent the means ± S.E., $n = 4$ different cell preparations.

**Effect of hypotonic buffer on Na+-dependent and Na+-independent taurocholate uptake.** Hepatocytes were exposed to isotonic HEPES buffer containing either Na+ (Na+ Buffer) or choline replacing Na+ (Choline Buffer) or the corresponding hypotonic buffer (Na+ or choline concentration reduced to 70 mM) for 25 min followed by determination of TC (20 μM) uptake. Na+-dependent uptake was calculated by subtracting uptake in the presence of Na+ from that in the presence of choline. Data represent the means ± S.E., $n = 4$ different cell preparations. *$, significantly different from respective control values.

In a previous study, we suggested that PKB, an effector of PI3K, may be involved in cAMP-mediated translocation of Ntcp (15). We therefore determined whether cell swelling also results in the activation of PKB. Exposure of hepatocytes to hypotonic media resulted in a time-dependent and wortmannin-sensitive activation of PKB, with significant activation observed at 5 min (Fig. 7). This result indicates that the activation of PKB by cell swelling is dependent on PI3K activity.
Effect of Cell Swelling on TC Uptake and p70S6K Activity—Another downstream effector of PI3K is p70S6K. Cell swelling also resulted in an activation of p70S6K (Fig. 8), as previously reported in hepatocytes (19). The effect on p70S6K was inhibited by wortmannin (data not shown), indicating dependence on PI3K. To determine whether the effect of cell swelling on TC uptake was mediated via p70S6K, we studied the effect of rapamycin, an inhibitor of p70S6K (29), on TC uptake and p70S6K activity. Rapamycin (200 nM) inhibited the increases in p70S6K activity induced by cell swelling but failed to inhibit the increases in TC uptake induced by cell swelling (Fig. 8). Rapamycin did not affect cell swelling-induced activation of PKB (data not shown). These results suggest that the effect of cell swelling on TC uptake is not mediated via p70S6K.

Effect of Cell Swelling on MAPK Activity—To determine whether the effect of cell swelling on TC uptake was mediated via the MAPK signaling pathway, we studied the effect of hypotonic media on MAPK activity and the effect of MAPK inhibitor (PD98059) on TC uptake. Exposure of hepatocytes to hypotonic media for 10 min failed to stimulate phosphorylation of MAPK substrate, Elk-1 (Fig. 9). We used 10 min of incubation, because hypotonic media has been reported to produce maximal activation of MAPK by 10 min in cultured hepatocytes (18). In contrast, HGF activated MAPK as evidenced by over...
2-fold increase in Elk-1 phosphorylation, and this effect was inhibited by a MAPK inhibitor, PD98059 (Fig. 9). To confirm this further, we determined time-dependent effect of hypotonic media on phosphorylation of p44/p42 (Erk1/Erk2) MAPK. Exposure of hepatocytes to hypotonic media failed to increase phosphorylation of either p42 or p44 MAPK for up to 25 min (Fig. 10), indicating that cell swelling under our experimental conditions did not activate MAPK. In addition, PD98059 failed to inhibit increases in TC uptake stimulated by cell swelling (Fig. 11). Taken together, these results indicate that the effect of cell swelling on TC uptake is not mediated via the MAPK signaling pathway.

DISCUSSION

The aim of the present study was to determine whether cell swelling affects hepatic uptake of bile acids and whether this effect is mediated via the PI3K and/or MAPK signaling pathway. Results suggest that cell swelling induced by hypotonic media stimulates Na+/TC cotransport by translocating Ntcp to the plasma membrane and that this effect is mediated via the PI3K signaling pathway as discussed below.

Cell swelling induced by hypotonic media rapidly stimulates TC uptake in hepatocytes in a time- and medium osmolarity-dependent manner (Figs. 1 and 2). The increase in TC uptake is due to an increase in Na+/TC cotransport (Fig. 3), indicating stimulation of Na+/TC cotransport. The increase in TC uptake is due to an increase in maximal transport velocity (Fig. 4), raising the possibility of an increase in the number of TC transporter in the plasma membrane. Because Ntcp mediates Na+/TC cotransport, we studied the distribution of Ntcp. Cell swelling was associated with an increase in plasma membrane Ntcp without changes in total cellular Ntcp. These results suggest that the increase in plasma membrane Ntcp is due to translocation of Ntcp instead of an increase in Ntcp synthesis. Taken together, these results suggest that the rapid stimulation of TC uptake by cell swelling results from an increase in plasma membrane Ntcp, which in turn results from a rapid translocation of Ntcp to the plasma membrane. These results are similar to the effect of cAMP on TC uptake and Ntcp translocation in hepatocytes (12, 13). Cell swelling induced by hypotonic media has previously been shown to increase biliary excretion of TC (18). The present study showing that cell swelling also activates TC uptake would indicate that transhepatic transport of TC is stimulated by cell swelling. Such a mechanism would be consistent with the stimulation of hepatic bile formation associated with cell swelling (30, 31).

Rapid regulation of solute transport by transporter translocation to the plasma membrane is well established for insulin-stimulated glucose transport (30). A similar mechanism of regulation has also been suggested for solute transport by hepatocytes. For example, cAMP rapidly stimulates Na+/TC cotransport by translocating Ntcp to the plasma membrane (13) and increases translocation of multi-drug-resistant proteins, Mrp2 and Mrp3, and SPGP (Sister of P-GlycoProtein canalicular bile acid transporter) to the canalicular membrane (33, 34). Canalicular multidrug resistance protein (Mrp2), which transports anionic conjugates like cysteinyl leukotrienes, also undergoes rapid and reversible translocation because of changes in cell hydration (35). Thus, transporter translocation appears to be a common mechanism for rapid
Regulation of solute transport in hepatocytes. Recent studies, however, suggest that extracellular ATP-dependent down-regulation of organic anion transport involves phosphorylation instead of translocation of OATP1 (36).

Our study also indicates that the effect of cell swelling on TC uptake is mediated via the PI3K signaling pathway. Cell swelling has been shown to activate PI3K in hepatocytes (19), as we have also observed in the present study. Wortmannin and LY294002, known inhibitors of PI3K (37, 38), inhibited cell swelling-induced increases in PI3K (data not shown), TC uptake (Fig. 5), and Ntcp translocation (Fig. 6). In a previous study we observed that wortmannin did not affect MAPK activity in hepatocytes (15). These results are consistent with the hypothesis that the effect of cell swelling is mediated via the PI3K signaling pathway. Cell swelling-induced increases in ATP release in a rat hepatoma cell line (21) and increases in ATP release and Cl− secretion in cholangiocytes (20) have also been suggested to be mediated via the PI3K signaling pathway. Our previous study suggested that cAMP-mediated stimulation of TC uptake and Ntcp translocation is also mediated via the PI3K signaling pathway (15). Cell swelling has also been shown to stimulate biliary bile acid secretion via the MAPK pathway (18). However, a recent preliminary study showed that cAMP-mediated stimulation of biliary TC secretion involves the PI3K pathway (34). This result raises the possibility that cell swelling-induced increases in biliary secretion of TC may also be mediated via the PI3K pathway. In addition, wortmannin has been shown to decrease bile acid secretion in isolated perfused rat livers (39). Thus, the PI3K signaling pathway may play an important role in the vectorial transport of bile acids across hepatocytes by stimulating both sinusoidal uptake and canalicular excretion.

Two downstream effectors of PI3K are PKB and p70S6K (40). PI3K phosphorylates lipids to produce phosphatidylinositol phosphates, such as phosphatidylinositol 3,4,5-triphosphate, which in turn affect cellular functions by allowing activation of PKB and p70S6K (41). This activation is dependent on the presence of PI3K products and requires sequential phosphorylation by phosphoinositide-dependent kinases (42). The regulatory effect of PI3K in insulin-stimulated translocation of glucose transporter (Glut4) has been proposed to be mediated via PKB (43, 44). In our study, the activation of both PKB and p70S6K by cell swelling was inhibited by wortmannin, indicating PI3K dependence. In addition, the effect on p70S6K was inhibited by rapamycin, an inhibitor of p70S6K (29). These results raise the possibility that either PKB and/or p70S6K may be the downstream effector. However, the effect of cell swelling on TC uptake does not appear to involve the PI3K/p70S6K pathway, because rapamycin failed to inhibit cell swelling-induced increases in TC uptake (Fig. 8). Recent studies showed that neither cAMP-stimulated TC uptake nor insulin-stimulated amino acid transport is mediated via the PI3K/p70S6K pathway (15, 45). Thus, based on the postulated role of PKB in glucose transporter translocation (43, 44), it is likely that the PI3K/PKB pathway is involved in cell swelling-induced stimulation of TC uptake and Ntcp translocation. The role of PKB could not be studied directly, because an inhibitor of PKB, like that of p70S6K, is not available. Transfection studies in a suitable cell line will be needed to further define the role of PKB.

Cell swelling has been proposed to stimulate biliary excretion of TC via the MAPK pathway (18). Cell swelling has been reported to stimulate MAPK in cultured hepatocytes (18) but not in freshly prepared hepatocytes (19). In our study, we used freshly prepared hepatocytes to study TC uptake, because TC uptake is down-regulated in cultured hepatocytes (46). As reported previously (19), cell swelling did not activate MAPK in freshly prepared hepatocytes. One reason for this difference may be that MAPK is already activated in freshly isolated hepatocytes. The presence of phosphorylated MAPK in control hepatocytes will be consistent with this view. However, HGF was able to further activate MAPK in this system. Thus, it is possible that hypotonicity, compared with HGF, is a weak stimulator of MAPK and hence did not produce further activation. In any event, because hypotonicity can still activate TC uptake, it is unlikely that the effect on TC uptake was mediated via the MAPK pathway. Furthermore, an inhibitor of MAPK kinase, PD98059, failed to affect cell swelling-induced increases in TC uptake. Thus, it is unlikely that the effect of hypotonicity on TC uptake and Ntcp translocation is mediated via the MAPK pathway. This, however, does not rule out a regulatory role of MAPK in Ntcp translocation.

Whether the effect of cell swelling on Ntcp translocation is a specific effect on TC transport or a general effect on transporters in hepatocytes is unclear. Considering that stimulation of Na+/TC cotransport would result in an increase in cell volume, a counterproductive effect during cell swelling and the subsequent RVD (28, 47), the latter possibility seems more likely. In fact, cell swelling is also associated with increased biliary excretion of cysteinyl leukotrienes and increased targeting of its transporter, MRp2, to the canalicular membrane (35, 48). It has been suggested that cell swelling-induced increases in ATP release may involve insertion of transporters in the plasma membrane (21). Other studies suggest that translocation of Ntcp is dependent on intact actin filament (15, 49), and cell swelling-dependent stimulation of biliary secretion of TC and cysteinyl leukotrienes is dependent on intact microtubules (48, 50). Furthermore, when exposed to hypotonic media, hepatocytes undergo a rapid increase in cell volume within 2–5 min, and this is followed by RVD, during which cell volume returns to near basal level within 15–20 min (28, 47). In the present study (Fig. 1), TC uptake did not increase significantly until 10 min, reaching a near maximum level at 25 min. These results may suggest that the activation of TC uptake coincides with the initiation of RVD and continues through RVD. Because exocytosis has been suggested to be increased during RVD (47), it is possible that cell swelling stimulates exocytosis by increasing vesicular trafficking along the cytoskeleton in an effort to decrease cell volume. This process results in the fusion of intracellular vesicles to the plasma membrane leading to plasma membrane translocation of various transporters stored in the intracellular vesicles. In summary, the present study shows that cell swelling induced by hypotonic media stimulates Na+/TC cotransport by translocating Ntcp to the plasma membrane, and this effect is mediated via the PI3K signaling pathway.

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