Short-term regulation of multidrug resistance-associated protein 3 in rat and human hepatocytes

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Chandra, Priyamvada, Peijin Zhang, and Kim L. R. Brouwer. Short-term regulation of multidrug resistance-associated protein 3 in rat and human hepatocytes. Am J Physiol Gastrointest Liver Physiol 288: G1252–G1258, 2005. First published January 13, 2005; doi:10.1152/ajpgi.00362.2004.—The short-term regulation of multidrug resistance-associated protein 3 (Mrp3/MRP3) by cAMP and PKC was investigated in sandwich-cultured rat and human hepatocytes and isolated perfused rat livers. The modulator glucagon (500 nM) and the phorbol ester PMA (0.1 μM) were utilized to increase intracellular cAMP and PKC levels, respectively. In glucagon-treated rat hepatocytes, efflux of the Mrp3 substrate 5-(6)-carboxy-2',7'-dichlorofluorescein (CDF) increased ~1.5-fold, even in hepatocytes treated with the organic anion transporter (Oatp) inhibitor sulfobromophthalein (SBP). Confocal microscopy revealed more concentrated Mrp3 fluorescence in the basolateral membrane (less diffuse staining pattern) with glucagon treatment. PMA had no effect on Mrp3 activity or localization in sandwich-cultured rat hepatocytes. Glucagon and PMA treatment in isolated perfused rat livers resulted in a threefold increase (14 ± 4.6 μl·min⁻¹·g liver⁻¹) and a fourfold decrease (1.3 ± 0.3 μl·min⁻¹·g liver⁻¹) in CDF basolateral clearance compared with control livers (4.7 ± 2.3 μl·min⁻¹·g liver⁻¹), whereas CDF biliary clearance was not statistically different. In sandwich-cultured human hepatocytes, glucagon treatment resulted in a 1.3-fold increase in CDF efflux and a concomitant increase in Mrp3 fluorescence in the basolateral membrane. In summary, cAMP and PKC appear to be involved in the short-term regulation of Mrp3/MRP3, as demonstrated by alterations in activity and localization in rat and human hepatocytes.

Traffic of transport proteins to and from the membrane has been shown to be dependent on short-term posttranscriptional regulation via second messengers (2, 39). cAMP, involved in activation of PKA, is an important second messenger that is responsible for the regulation of many cellular processes, ranging from control of various metabolic events, muscle contraction, secretion, memory, and cell growth to protein translocation (32). Other signaling molecules, such as PKA, PKB, PKC, and phosphatidylinositol 3-kinase (PI3K), also have been implicated in basal and cAMP-mediated trafficking pathways (37).

Regulation of hepatic canalicular transport proteins via signaling pathways has been investigated previously. Taurocholate and the cell-permeable cAMP analog 2'-O-dibutyryl-cAMP (DBcAMP) increased multidrug resistance proteins 1 and 2 (Mdr1 and Mdr2), Mrp2, and Bsep protein content in rat canalicular membrane vesicles 1.5- and 3-fold, respectively, and also significantly increased transport activity (7). An increase in bile acid secretion and a concomitant increase in the canalicular membrane circumference have been observed as a result of DBcAMP stimulation in hepatocyte couplets (29). These results suggest that stimulation of biliary excretion is related to increased insertion of transport protein-containing vesicles into the apical membrane. Recently, the trafficking of Bsep has been shown to be dependent on the basal activity of PKC and p38MAPK (13).

Most short-term regulation studies of hepatic basolateral transport proteins have focused on Ntcp. Pretreatment with DBcAMP resulted in an ~1.3-fold increase in taurocholate uptake in rat hepatocytes and HepG2 cells (25). Although cAMP also can affect gene transcription and, hence, increase transport protein, cycloheximide (an inhibitor of protein synthesis) did not inhibit cAMP-induced increases in taurocholate uptake by Ntcp (25). Cellular fractionation of CAMP-treated hepatocytes revealed trafficking of Ntcp from endosomes to the basolateral membrane (25). In the same study, 4β-phorbol 12-myristate 13-acetate (PMA), a PKC stimulator, inhibited DBcAMP-induced stimulation of taurocholate uptake via Ntcp, thus implicating PKC in the downregulation of this transporter. In addition to cAMP-dependent trafficking of proteins from endosomal compartments to basolateral or apical membranes, cAMP also may modulate protein activity by altering the phosphorylation state of the transporter. A loss of organic anion transporter (Oatp1a1)-mediated transport resulted from increased phosphorylation. However, this was not due to a change in localization, because internalization of the protein did not occur (8).

Protein regulation can occur at the level of transcription, translation, and posttranslation. Gene transcription regulation of transport proteins [e.g., Na+-taurocholate cotransporting polypeptide (Ntcp), bile salt export pump (Bsep), and multidrug resistance-associated proteins 2 and 3 (Mrp2 and Mrp3)] by nuclear hormone receptors [e.g., farsenoid, liver, and pregnane receptors (FXR, LXR, and PXR)] has received much attention recently (15). Several studies also have examined the short-term regulation and trafficking (i.e., posttranslational modifications) of hepatic transport proteins (13, 20, 36). The exact mechanisms for short-term regulation of membrane transport may differ; however, they generally fall into two categories: alterations in transporter function and changes in the number of transporters in the membrane. Because these regulatory effects generally occur very rapidly, increased transcription or translation is usually not the cause.

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Mrp3/MRP3, a member of the multidrug resistance-associated protein family, is localized to the basolateral membrane of hepatocytes. Normally expressed at low basal levels, Mrp3/MRP3 is highly inducible and is responsible for mediating the efflux of organic anions, bile acids, and glucuronide and glutathione conjugates from the liver into sinusoidal blood (10, 26). The mediators responsible for regulating the short-term activity of Mrp3/MRP3 have not been reported.

In the studies described here, rat and human sandwich-cultured hepatocytes were used to investigate the short-term regulation of Mrp3/MRP3. The TR− rat strain, which is hereditarily deficient in Mrp2, was utilized for the hepatocyte experiments. The high level of hepatic basolateral Mrp3 (a compensatory mechanism for the lack of Mrp2 transport capacity into bile) makes TR− rat hepatocytes an ideal model for studying the regulation of this transport protein (27). In addition, transport of overlapping substrates by canalicular Mrp2 is not a confounding factor when disposition data from TR− rats are interpreted. To compare results from hepatocyte studies with those from a more complex organ system, experiments were also carried out in isolated perfused rat livers (IPL). The effects of the second-messenger cAMP and PKC were assessed by measuring alterations in trafficking and activity of Mrp3/MRP3. The hormonal modulator glucagon (a G protein-coupled receptor agonist) was used to elevate intracellular cAMP, whereas PMA served to increase PKC levels.

MATERIALS AND METHODS

Chemicals. 5-(6)-Carboxy-2′,7′-dichlorofluorescein (CDF) and CDF diacetate were obtained from Molecular Probes (Eugene, OR); collagenase (type I, class I) from Worthington Biochemicals (Freehold, NJ); Dulbecco’s modified Eagle’s medium (DMEM) and insulin from GIBCO (Grand Island, NY); 10× DMEM, penicillin-streptomycin solution, fetal bovine serum (FBS), glucagon, 3-isobutyl-1-methylxanthine (IBMX), DBcAMP, PMA, and sulfobromophthalein (BSP) from Sigma Chemical (St. Louis, MO); and ITS culture supplement (Bedford, MA). All other chemicals and reagents were of analytic grade or higher and were readily available from commercial sources.

Animals. Male Wistar rats (250–330 g; Charles River Laboratories, Raleigh, NC) and male Mrp2-deficient (TR−) rats bred in the University of North Carolina School of Pharmacy animal facility (250–330 g; originally obtained from Dr. Mary Vore, University of Kentucky, Lexington, KY) were used for IPL studies and for isolation of hepatocytes from whole liver, respectively. Rats were kept in a constantly alternating 12:12-h light-dark cycle, with access to food and water ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee.

Hepatocyte isolation. Isolation and culturing of TR− rat hepatocytes were performed according to previously described methods (6). Briefly, after the rat was anesthetized with ketamine (60 mg/kg ip) and xylazine (12 mg/kg ip), the portal vein and inferior vena cava were cannulated and the liver was perfused with a Ca2+-free buffer equilibrated with 95% O2–5% CO2 at 30 ml/min. After 10 min of perfusion in a recirculating manner, collagenase (0.05–0.075 g) was added to the perfusate reservoir. The liver was perfused for a total of 10 min with collagenase buffer, removed from the rat, and immersed in supplemented DMEM. Hepatocytes were filtered through a 70-μm mesh filter and then centrifuged. The pellet was resuspended in equal parts of medium and isotonic Percoll (90%) and centrifuged to separate nonviable cells. A final centrifugation step was performed to remove any remaining Percoll from the hepatocytes. Viability was >90% as determined by trypan blue exclusion with a typical yield of 2–3×10^6 cells. Cells were resuspended in medium and diluted to a final concentration of 1×10^6 cells/ml.

Sandwich-cultured hepatocytes. Permanox petri dishes (60 mm) coated with 0.2 ml of ice-cold neutralized type I collagen solution were used for maintenance of cultures. At −1–2 h after seeding at 3×10^6 cells/dish, medium was replaced with 3 ml of warm, fully supplemented DMEM (4 mg/l insulin, 0.1 μM dexamethasone, and 5% FBS); 24 h later, the hepatocytes were overlaid with 0.2 ml of ice-cold neutralized type I collagen solution and allowed to gel at 37°C. After 45 min, 3 ml of FBS-free DMEM (0.1 μM dexamethasone and 1% ITS) were added to each dish and replaced daily. Rat and human hepatocytes were cultured for 4 and 6 days, respectively, before experimentation. Hepatocytes used for immunohistochemical analysis by confocal microscopy were treated with vehicle, 500 nM glucagon + 500 μM IBMX (20 min), or 0.1 μM PMA (60 min) and then fixed with 2 ml of ice-cold acetone for 10 min at 4°C before storage at −80°C. IBMX was used to inhibit phosphodiesterase activity and minimize breakdown of cAMP during the incubation period. Human hepatocytes, isolated by a previously described method (19), were seeded at 4×10^6 cells/dish onto 60-mm collagen-coated Permanox dishes and maintained as described above. Glucagon-treated hepatocytes were lysed with 2 ml of 0.1 N HCl and gently shaken for 20 min at room temperature; then intracellular cAMP levels were determined using a radioimmunoassay of acetylated cAMP (17).

Confocal microscopy. Sandwich-cultured hepatocyte samples were thawed in phosphate-buffered saline (PBS) and subsequently incubated in blocking buffer (5% goat serum and 1% BSA) for 45 min at room temperature. Samples were incubated with primary Mrp3 (kindly donated by Dr. Yuichi Sugiyama, Tokyo, Japan), MRP3 (Alexis Biochemicals, San Diego, CA), or dipeptidyl peptidase IV (DPPIV; Serocte, Raleigh, NC) antibody in PBS with 1% BSA for 60 min. Subsequently, samples were washed with 1 ml of PBS three times for 10 min and incubated with Alexa Fluor 488-conjugated goat anti-mouse IgG or Alexa Fluor 488-conjugated goat anti-rabbit IgG.
cultured TR

Fig. 2. CDF efflux at 10 min expressed as percentage of control (Ctrl) in 500 nM glucagon + IBMX-treated (Gluc), 50 μM bromosulfophthalein (BSP)-treated, and 500 nM glucagon + IBMX and BSP-treated (G+B) sandwich-cultured TR− rat hepatocytes on day 4. Values are means ± SD (n = 3 livers in triplicate). *P < 0.01 vs. control. *P < 0.01 vs. BSP.

(Molecular Probes) in PBS for 60 min. Samples were again washed in PBS three times for 10 min and then mounted onto coverslips using Permount histological mounting medium. For the blank negative control, the sample was treated as described above, except the primary antibody staining was omitted. Approximately 8–10 fields were observed before representative fluorescent images were obtained with an upright laser scanning confocal microscope (model SP2 AOBs, Leica Microsystems, Mannheim, Germany). The laser excitation power, pin hole, and detector sensitivity settings of the microscope were kept constant during observation of control and modulator-treated samples.

Transport experiments. Efflux studies were conducted in triplicate in sandwich-cultured rat hepatocytes. Cells were rinsed twice with 2 ml of Hank's balanced salt solution (HBSS) and preincubated in 2 ml of HBSS with vehicle, 500 nM glucagon + 500 μM IBMX (20 min), 100 μM DBcAMP + 500 μM IBMX (60 min), or 100 nM PMA (60 min) at 37°C. Subsequently, cells were incubated in 2 ml of 10 μM CDF diacetate in HBSS with vehicle or modulator for 10 min and washed three times with 3 ml of ice-cold HBSS. CDF transport was reinitiated by incubation of cells in warm HBSS at 37°C (efflux phase). Efflux medium was sampled after 10 min and analyzed for CDF by fluorescence spectroscopy using a microplate fluorescence reader (model FL600, Bio-Tek Instruments, Winooski, VT) at wavelengths of 505 nm (excitation) and 523 nm (emission). Preliminary experiments were conducted to confirm that the modulators did not affect steady-state concentrations of CDF during the 10-min incubation with 10 μM CDF diacetate. In conditions where BSP was utilized, 50 μM BSP was present in the 10-min efflux phase. The hepatocytes were lysed with 2 ml of 0.5% Triton X-100 solution and gently shaken for 20 min at room temperature. CDF efflux was normalized to total protein in cell lysates using the bicinchoninic acid method with albumin standard (30).

IPL studies. Male Wistar rats were anesthetized with ketamine (60 mg/kg ip) and xylazine (12 mg/kg ip), the liver was isolated, and the bile duct and portal vein were cannulated and perfused in situ in a single-pass manner using previously described techniques (4). After the liver was transferred to a 37°C perfusion chamber, the liver and perfusate were allowed to equilibrate for 15 min before perfusion with 1 μM CDF in oxygenated 37°C Krebs-Ringer bicarbonate buffer containing 16.5 μM taurocholate and vehicle, 500 nM glucagon + 500 μM IBMX, or 0.01 μM PMA for 35 min. The perfusate then was switched to CDF-free buffer (containing vehicle, 500 nM glucagon + 500 μM IBMX, or 0.01 μM PMA) for 25 min. Bile and perfusate samples were collected from 0 to 60 min and frozen at −20°C until analyzed by fluorescence spectroscopy as described above. Perfusion pressure and bile flow were used to assess liver viability. To determine hepatic CDF concentrations, the mass of CDF in the liver at each time interval (determined by mass balance) was divided by 0.7 ml/g liver, the intracellular volume of the liver (35). Basolateral clearance (ClBL) values were calculated as the ratio of the total amount of CDF excreted into perfusate from 35 to 60 min to the area under the curve of CDF in the liver over this time interval. Biliary clearance values were calculated as the ratio of the total amount of CDF excreted into bile from 35 to 60 min to the area under the curve of CDF in the liver over this time interval.

Data analysis. CDF efflux data from sandwich-cultured rat hepatocyte experiments were determined in triplicate from three rat livers per group. Four rats per group were used in the IPL studies. Values are means ± SD. Statistically significant differences were determined by analysis of variance and appropriate post hoc multiple comparison procedures. Statistical significance was determined at α = 0.05.

RESULTS

The effect of glucagon on cAMP in culture was determined to confirm that the hormonal modulator was enhancing CAMP levels within the hepatocytes. Intracellular cAMP was significantly higher on day 4 in sandwich-cultured rat hepatocytes treated with 500 nM glucagon + 500 μM IBMX than in vehicle controls: 967 ± 243 vs. 1.9 ± 0.02 pmol/106 hepatocytes. CDF efflux into the extracellular medium from glucagon-treated cells was 1.3- to 1.8-fold higher than from control cells (Fig. 1A; P < 0.01). To confirm that this effect was specifically due to increased intracellular levels of cAMP, cells were incubated with the cell-permeable cAMP analog DB-cAMP. CDF efflux increased ~1.4-fold: from 395 ± 23.4 in control to 557.8 ± 27.4 in DBcAMP-treated hepatocytes. PMA

Fig. 3. Representative immunofluorescence confocal microscopy images of dipeptidyl peptidase (left; red) and multidrug resistance-associated protein 3 (Mrp3, middle; green) and both images overlaid (right) in sandwich-cultured TR− rat hepatocytes on day 4. Scale bar, 10 μm.

AJP-Gastrointest Liver Physiol • VOL 288 • JUNE 2005 • www.ajpgi.org
treatment did not affect CDF efflux relative to control cells (Fig. 1B).

CDF efflux from the basolateral membrane of rat hepatocytes is hypothesized to be mediated by Mrp3 and, possibly, Oatp1a1/1b2 hepatic transport proteins. To minimize Oatp-mediated CDF transport, hepatocytes were incubated with the competitive Oatp inhibitor BSP during the efflux phase. CDF accumulation in the extracellular medium at 10 min decreased to ~55% (P < 0.01) of control in the presence of BSP, suggesting that Oatp proteins were at least partially inhibited. However, in glucagon-treated hepatocytes with BSP in the efflux phase, CDF efflux increased from ~55% to ~85% of control (P < 0.01; Fig. 2).

Confocal microscopy was used to assess possible alterations in Mrp3 localization to the basolateral membrane. Sandwich-cultured TR− rat hepatocytes coincubated with antibodies against Mrp3 and the apical marker protein DPPIV revealed two distinct staining patterns after overlay (Fig. 3). Both proteins were present intracellularly; however, Mrp3 was also present on the basolateral membrane encircling the cell. DPPIV was localized to the canalicular surface, appearing as tubular structures surrounding the basolateral membrane.

Confocal images of vehicle-treated TR− rat hepatocytes revealed Mrp3 fluorescent staining intracellularly and in a diffuse pattern on the basolateral membrane (Fig. 4). Hepatocytes treated with 500 nM glucagon + 500 µM IBMX displayed a less diffuse fluorescent pattern on the basolateral membrane, consistent with increased basolateral trafficking of Mrp3 compared with control (Fig. 4).

In IPL studies with Wistar rats, glucagon treatment resulted in a significant increase in CDF ClBL (14 ± 4.6 µl·min⁻¹·g liver⁻¹), whereas treatment with the PKC activator PMA resulted in a significant decrease in ClBL of CDF (1.3 ± 0.3 µl·min⁻¹·g liver⁻¹) relative to control values (4.7 ± 2.3 µl·min⁻¹·g liver⁻¹, P < 0.05; Fig. 5A). CDF biliary clearance was unaltered after glucagon or PMA treatment (Fig. 5B).

CDF efflux increased 1.3-fold (P < 0.04) in human hepatocytes pretreated with glucagon + IBMX, consistent with increased MRP3 activity (Fig. 6). Confocal microscopy images of MRP3 in vehicle-treated sandwich-cultured human hepatocytes revealed a staining pattern similar to that observed in rat hepatocytes (Fig. 7). Fluorescence, representing MRP3, was increased on basolateral membranes of human hepatocytes treated with glucagon and was less diffuse than in control, consistent with data obtained in sandwich-cultured rat hepatocytes.

**DISCUSSION**

The short-term regulation of hepatic transport proteins is controlled by intracellular signaling pathway mediators. In this study, we investigated the effects of cAMP and PKC on Mrp3/MRP3 activity and localization in rat and human hepatocytes. The impact of elevated intracellular cAMP and PKC levels mediated by the G protein-coupled receptor agonist glucagon and the PKC activator PMA (a phorbol ester) on...
Mrp3/MRP3 localization and CDF efflux, respectively, was determined. The incubations with glucagon and PMA were short-term (<1 h); therefore, any effects may be attributed to acute changes in transporter activity and localization and not to alterations at the gene expression level.

Glucagon is a 29-residue peptide hormone that is responsible for the breakdown of glycogen in the liver. As a G protein-coupled receptor agonist, it binds to receptors on the hepatocyte cell surface and results in formation of a second messenger, cAMP, as part of its signaling cascade, which leads to the release of glucose. This hormonal modulator was utilized because it has a short duration of action (i.e., ~20 min) and because hepatocytes have many glucagon receptors on their cell surface (32).

CDF is transported into rodent hepatocytes by Oatp1a1/1b2 and is excreted across the canalicular membrane by Mrp2 and across the basolateral membrane by Mrp3 (38, 40). Small interfering RNA knockdown of Mrp3 resulted in increased canalicular excretion of CDF by Mrp2 (34), thus substantiating the use of CDF as an Mrp3 probe. In sandwhich-cultured hepatocytes from TR− rats, CDF is an ideal substrate for measuring Mrp3 activity, inasmuch as canalicular Mrp2 is not present to compete with Mrp3 for hepatic excretion of CDF from the basolateral surface.

Hepatic Mrp3 activity, determined by measuring basolateral CDF efflux from sandwich-cultured TR− rat hepatocytes, increased 1.3- to 1.8-fold with glucagon and DBcAMP pretreatment relative to control (Fig. 1). Inasmuch as CDF transport in rat hepatocytes has been shown to occur by multiple mechanisms (40), hepatocytes were incubated with the Oatp1a1/1b2 inhibitor BSP (12) to minimize Oatp-mediated CDF transport during the efflux phase of the experiment. BSP was included to inhibit Oatp-mediated reuptake of CDF from the extracellular medium after efflux from the cells. By elimination of CDF reuptake, the Mrp3 efflux process could be assessed accurately. Surprisingly, CDF efflux decreased to ~55% of control in the presence of BSP (Fig. 2). Oatp1a1 has been reported to be a bidirectional transporter (18). Therefore, it is possible that BSP inhibited Oatp1a1-mediated efflux of CDF. Alternatively, BSP may have partially inhibited Mrp3-mediated efflux of CDF, although BSP has not been reported to be an Mrp3 substrate or inhibitor. However, given that this compound is a substrate for Mrp2 and that Mrp2 and Mrp3 share overlapping substrate specificity, it is possible that BSP reduced CDF basolateral efflux via competitive inhibition of Mrp3 (5, 16).

In glucagon-treated hepatocytes, CDF efflux was reduced to only ~85% of control levels in the presence of BSP. Together, these data demonstrate a ~1.5-fold increase in CDF efflux after glucagon pretreatment (irrespective of the presence of BSP). This increased CDF efflux after glucagon pretreatment should represent efflux by basal (i.e., Mrp3 present on the membrane before glucagon treatment) and trafficked Mrp3 protein, although the involvement of other Mrp proteins cannot be ruled out on the basis of these functional data alone.

The increase in Mrp3 transport activity in glucagon-treated sandwich-cultured TR− hepatocytes likely was due, in part, to an increase in the amount of Mrp3 protein on the basolateral membrane, as demonstrated by confocal microscopy. As shown in Fig. 3, Mrp3 appeared within the cytosol, presumably in intracellular vesicles, and also on the basolateral membrane. The apical marker protein DPPIV was present within the canalicular networks (apical membrane), which appeared as tubular structures surrounding the hepatocytes, and within the cell. The less diffuse and more defined fluorescent staining pattern of Mrp3 on the basolateral membrane after glucagon treatment suggested that elevated cAMP levels may have resulted in the trafficking of Mrp3 from intracellular stores to the basolateral membrane.

IPL were treated with glucagon + IBMX to determine whether findings in the whole organ system would be similar. Treatment with glucagon resulted in a three-fold increase in Mrp3 activity, as determined by ClBL of CDF, consistent with

![Fig. 6. CDF efflux at 10 min in control and 500 nM glucagon + IBMX-treated sandwich-cultured human hepatocytes on day 6. Values are means ± SD (n = 2 livers in triplicate). Each symbol represents results from an individual liver preparation. *P < 0.04 vs. control.](http://ajpgi.physiology.org/)

![Fig. 7. Representative immunofluorescence confocal microscopy images of MRP3 (red) in control (left) and 500 nM glucagon + IBMX-treated (right) sandwich-cultured human hepatocytes on day 6. Scale bar, 25 μm.](http://ajpgi.physiology.org/)
the results from the sandwich-cultured hepatocyte experiments. Treatment with the PKC activator PMA had the opposite effect on C_{H2}, resulting in a nearly four-fold decrease relative to vehicle-treated IPL. This latter result is in agreement with PKC stimulators attenuating cellular uptake by rat organic anion transporter 3 (Oat3) by decreasing the V_{max} of this protein (33). These results suggest that PKC may be involved in the short-term downregulation of hepatic Mrp3 in rodents, although it is unclear why PMA had no effect in sandwich-cultured hepatocytes. The literature suggests that suppression (9, 22) and stimulation (21, 28) of transport activity by PKC can occur. This fact, along with the biochemical differences that may exist between sandwich-cultured hepatocytes and IPL (e.g., alterations in cellular physiology and different protein expression levels), may account in part for the discrepancy in the role of PKC in these two systems. Several isoforms of classical and novel PKC are regulated by diacylglycerol; thus, differential expression of PKC isoforms is possible in cultured cells relative to in vivo conditions (31). Therefore, the particular PKC isoform(s) that was activated by the diacylglycerol analog (PMA) and was responsible for the decreased Mrp3 activity in the IPL study may not have been sufficiently expressed in cultured hepatocytes. Another potential reason for the different effect of PKC on Mrp3 activity in the two model systems may be that the appropriate intracellular concentration of PMA for activation of the necessary isoform(s) may not have been reached in cultured hepatocytes.

Interestingly, glucagon treatment of sandwich-cultured human hepatocytes had a similar effect on CDF efflux (3.1-fold increase) and Mrp3 trafficking as in the rat hepatocytes. This suggests that cAMP is involved in the short-term regulation of MRP3 in humans as well as rodents.

Although the effect on Mrp3/MRP3 activity and localization demonstrated in this study appeared to be a direct result of elevated cAMP, it is likely that other signaling molecules also were involved in mediating this event(s). cAMP has been shown to activate several intracellular messengers and kinases such as Ca^{2+}, PKA, PKC, PI3K, and MAPK, all of which have been implicated in regulating some aspect of hepatic transport (1). These signal transduction pathways are complicated, and undoubtedly other “players” are involved in mediating the downstream effects of cAMP on Mrp3. For example, PI3K has been implicated in the regulation of ATP-binding cassette transporters, inasmuch as it has been shown to modulate vesicular trafficking of these proteins (23). In addition, PI3K lipid products were required for maximal Bsep and Mrp2 transport activity in the canalicular membrane (24).

Glucagon was expected to alter Mrp2 activity via elevation of cAMP, as shown in previous cAMP modulation studies (29). However, no functional increase in Mrp2 activity was observed in the IPL experiments. One possible explanation for this apparent discrepancy may be that the increase in intracellular cAMP due to glucagon treatment was a localized cellular phenomenon that only occurred near the region of the hepatic basolateral membrane. Levels of cAMP near the canalicular membrane and, hence, near the subapical compartments in which Mrp2 resides may not have been elevated; therefore, no increase in Mrp2 activity due to trafficking and insertion into the canalicular membrane was observed. Alternatively, the region-specific localization of specific phosphodiesterases and regulatory subunits of cAMP-dependent protein kinases may assist in the selective regulation of cellular function by cAMP. This may have been another contributing factor to the lack of effect of cAMP on Mrp2 activity in the canalicular membrane (11, 14).

In summary, it is not surprising that pretreatment of sandwich-cultured hepatocytes with glucagon, by increasing cellular cAMP, resulted in an increase in Mrp3/MRP3 activity in rat and human hepatocytes. One mechanism responsible for this increase in activity appears to be enhanced translocation from intracellular compartments to the basolateral membrane. The PKC activator PMA appeared to decrease the activity of Mrp3 in IPL. Bile acids are known to affect signaling mediators such as cytosolic Ca^{2+}, PKA (cAMP-dependent protein kinase), and PKC (3). Therefore, it is plausible that when hepatocellular bile acid levels are elevated, the appropriate signal transduction pathway is activated to translocate Mrp3 to the basolateral membrane in an effort to reduce the bile acid load on the cell.

Short-term modulation of proteins is a highly regulated process that potentially can influence hepatic transport and the hepatobiliary disposition and systemic exposure to xenobiotics and metabolites. Understanding the basic regulation of transport proteins will increase knowledge of hepatic transport biology and enable predictions of how compounds and/or disease states that affect intracellular regulatory mechanisms may cause alterations in drug transport.

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