Marinobufagenin (MBG) is an endogenous mammalian cardiotonic steroid involved in the inhibition of Na\(^+/\)K\(^+/\)-ATPase. Increased plasma levels have been reported in patients with volume expansion-related hypertension. We have recently demonstrated that MBG impairs first trimester cytotrophoblast (CTB) cell proliferation, migration, and invasion, which may play a role in the development of preeclampsia. However, whether apoptosis contributes to altered CTB cell function by MBG remains unknown.

Using the human extravillous CTB cell line SGHPL-4, we examined the effect of MBG and a similar Na\(^+/\)K\(^+/\)-ATPase inhibitor, ouabain, on the phosphorylation status of Jnk, p38, and Src. Additionally, we measured apoptosis by caspase 9 and 3/7 activity and by annexin-V staining. We also investigated interleukin-6 (IL-6) secretion with or without p38 and Jnk inhibition. MBG significantly increased the phosphorylation of Jnk, p38, and Src and increased the expression of caspase 9 and 3/7 indicating the activation of apoptosis. MBG treatment also stimulated the expression of the early apoptosis marker, annexin-V, which was prevented by Jnk and p38 inhibition. MBG also stimulated the secretion of IL-6, which was attenuated by p38 inhibition. Ouabain had similar effects to those of MBG, suggesting that the apoptotic effects on CTB cells may be mediated by inhibition of Na\(^+/\)K\(^+/\)-ATPase. In conclusion, the MBG-induced impairment of CTB function occurs via activation of Jnk, p38, and Src leading to increased apoptosis and IL-6 secretion. These observations may have clinical applicability with respect to the therapy of preeclampsia.

Marinobufagenin (MBG)\(^2\) is an endogenous mammalian cardiotonic bufadienolide that inhibits the membrane-bound sodium pump Na\(^+/\)K\(^+/\)-ATPase and also has vasconstrictive properties (1–4). Circulating levels of MBG have been reported to be increased in patients with volume expansion-mediated hypertension and preeclampsia (5–8). Preeclampsia is a syndrome that occurs in 3–10% of pregnancies (9, 10) and is a leading cause of maternal and fetal morbidity and mortality (9). Preeclampsia is characterized by the de novo development of hypertension and proteinuria after 20 weeks of gestation (9, 11). The etiology of this syndrome remains unknown.

In normal pregnancy, placental development depends upon the differentiation of fetus-derived epithelial cells called cytotrophoblasts (CTBs), which then differentiate into an invasive cell population, termed extravillous CTBs. CTB invasion is a critical step for proper placental establishment and initiates a cascade of events leading to the remodeling of maternal vessels, which is necessary for proper fetal perfusion (12–16). Preeclampsia is associated with shallow placentation, inadequate invasion, and insufficient remodeling of the maternal arteries (13, 15). In an animal model of preeclampsia, we have shown that urinary excretion of MBG is elevated prior to the development of hypertension indicating that it may play a key role in the pathogenesis of preeclampsia (7). In addition, administration of an anti-MBG antibody corrected the deoxycorticosterone acetate-salt hypertension in pregnant rats, which demonstrated elevated urinary excretion of MBG (7, 8). These findings suggest that MBG might affect first trimester CTB function. Recently, we demonstrated that MBG impairs the proliferation, migration, and invasion of CTB cells (17, 18). However, the underlying mechanisms by which these alterations occur remain unknown.

Activation of mitogen-activated protein kinase (MAPK) signaling is known to play a critical role in the regulation of cellular proliferation and differentiation (19–25). In general, activated ERKs control cell proliferation and differentiation (19–21). On the other hand, stimulated Jnk and p38 pathways regulate cell proliferation, invasion, survival, migration, growth arrest, and apoptosis (19, 21–25). The particular function regulated by MAPKs is likely to depend on the cell type, the stimulus, and the duration and strength of kinase activities. Activation of AKT increases cellular proliferation and protection from apoptosis through phosphorylation and inactivation of several effectors, including Bad and caspase 9 (26–28). Several studies have implicated caspasases (cysteine aspartate-specific proteases) as the molecular instigators of apoptosis (29–30).

Recent studies have shown that cardiotonic steroids may have variable influences on cell proliferation, differentiation, and eventually cell death via Na\(^+/\)K\(^+/\)-ATPase signaling pathways (31). In tumor cells, high concentrations of bufalin induce apoptosis, which is associated with an increase in intracellular Na\(^+\) because of inhibition of the Na\(^+/\)K\(^+/\)-ATPase (32). In addition, bufalin induces apoptosis in endometrial cells, which is
associated with a down-regulation of the cell cycle and anti-apoptotic proteins Bcl-2 and Bcl-XL and the concomitant activation of Bax and caspase 9 (33).

Interleukin-6 (IL-6) is a multifunctional cytokine exhibiting numerous functions, which include the regulation of proliferation and differentiation in a variety of cells (34–37). It has been demonstrated that the major signal transduction pathways induced by IL-6 involve p38 (38). It has also been postulated that IL-6-induced signal transduction pathways lead to apoptosis of 1A-9-M cells (39) and that the Src family of kinases also likely regulate p38-mediated IL-6 production following hypoxia in Kupffer cells (40).

Recently, evidence has accumulated that indicates that Na+/K+-ATPase can function as a transmembrane signal transducing complex (i.e. signalosome) (1, 41). Cardiotoxic steroids, including MBG and ouabain, another Na+/K+-ATPase inhibitor, can increase intracellular Ca2+, increase reactive oxygen species production, and activate Src, phosphatidylinositol 3-kinase/Akt, and NFκB (31). Thus, cardiotoxic steroids can modify a number of cellular functions, such as cellular proliferation, via their effects on Na+/K+-ATPase in addition to their known Na+/K+-ATPase-dependent and -independent cardiovascular effects. However, very little is known about MBG-mediated Na+/K+-ATPase intracellular signaling effects on CTB function.

Therefore, we examined the effects of MBG on Jnk, p38, and Src phosphorylation, the early apoptosis markers caspases 9 and 3/7, and annexin-V staining to determine whether MBG causes apoptotic signaling in CTB cells. In addition, we examined IL-6 secretion in response to MBG and determined whether apoptosis and IL-6 secretion could be prevented by the inhibition of p38 and Jnk. We determined that the pro-apoptotic effect of MBG and ouabain on CTB cell function was mediated by Jnk, p38, and Src pathways and by the stimulation of IL-6 production. The similarity of the effects of MBG and ouabain on CTB apoptosis suggests a common pathway and that Na+/K+-ATPase inhibition may play a role.

**EXPERIMENTAL PROCEDURES**

*Cell Culture*—The human extravillous CTB cell line SGHPL-4 utilized in these studies was derived from first trimester chronic villous tissue (41) and was kindly provided by Dr. Guy Whitley (St. George’s Hospital Medical School, London, UK). These cells are well characterized and share many characteristics with isolated primary cells, including the expression of cytokeratin-7, HLA class I antigen, HLA-G, BC-1, CD9, human chorionic gonadotropin, and human placental lactogen (42–45).

SGHPL-4 cells were cultured in Ham’s F-10 nutrient mix supplemented with 10% fetal bovine serum, penicillin G (100 units/ml), streptomycin (100 mg/ml), and 2 mm l-glutamine (Sigma). Cells were incubated at 37 °C, 5% CO2, and 99% humidity (Fisher, Isotemp CO2 incubator).

**Effect of MBG and Ouabain on Jnk, p38, and Src Phosphorylation**—MBG (99.5% purity) was purified from parotid glands of *Bufo marinus* toads as reported previously (46). The effect of MBG on Jnk, p38, and Src phosphorylation was evaluated by the cellular activation of signaling ELISA (CASE) kit (SuperArray) (47). The kit includes a complete antibody-based detection system for colorimetric quantification of the relative amount of phosphorylated protein and total target protein. For the CASE assay, SGHPL-4 cells were seeded into 96-well plates and were stimulated with 10 or 100 nM MBG or ouabain for 10, 30, 60, 120, and 240 min. Detection of total and phosphorylated protein expression was determined according to the manufacturer’s protocol.

**Effect of MBG and Ouabain on Caspase 9 and Caspase 3/7 Activity**—The Apo-ONE homogeneous caspase 9 and caspase 3/7 assays were performed in 96-well formats after treatment of the cells with 10 nM MBG or 10 nM ouabain for 2, 4, or 6 h. One hundred μl of Apo-ONE caspase 9 or caspase 3/7 (Promega) reagent was added to each well. After 1 h of incubation at 37 °C, the luminescence was measured by a luminometer (Fluoroskan Ascent FL, Thermo Labsystems) for the evaluation of caspase 9 and 3/7 activity as described previously (30, 32, 48).

**Annexin-V Staining**—Annexin-V is a phospholipid-binding protein with a high affinity for phosphatidylserine. During apoptosis, phosphatidylserine translocates to the outer surface of apoptotic cells. Detection of cell-surface phosphatidylserine with annexin-V thus serves as a marker for apoptotic cells (49). CTB cells were seeded on coverslips in a 6-well culture plate and allowed to adhere overnight. CTB cells were stimulated with 10 or 100 nM MBG or ouabain for 24 h. Some cells were pretreated for 2 h with p38 (SB 202190, Sigma) or Jnk (SP 600125, Invitrogen) inhibitors before treatment with 10 and 100 nM MBG or ouabain for 24 h. Coverslips were removed and placed in a new 6-well plate with cold incubation buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, and 5 mM CaCl2). Coverslips containing cells were gently washed three times for 5 min with incubation buffer. Cells were then incubated for 30 min in 1:50 dilution of biotinylated annexin-V (Roche Applied Science) in incubation buffer. After incubation, cells were gently washed three times for 10 min in incubation buffer and then fixed in 4% paraformaldehyde for 10 min at room temperature. Cells were rinsed three times for 5 min in PBS and incubated with 1:200 dilution of Cy3-labeled streptavidin (GE Healthcare) (diluted in PBS) for 30–60 min at room temperature. After incubation, cells were washed three times for 10 min with PBS and mounted by coverslips onto microscope slides along with the nuclei marker 4’,6’ diamidino-2-phenylindole (Invitrogen). Cells were visualized using an Olympus Fluoview FV 300 confocal laser-scanning microscope.

**Cytokine Antibody Array Assay**—The cytokine antibody array (Panomics) is based on the sandwich ELISA method for detecting protein (50). CTB cells were treated with 10 and 100 nM MBG or ouabain in serum-free medium. After 24 h treatment, media were collected from the flasks seeded with CTB cells. Cells treated with DMSO were used as controls.

The samples were collected from cells treated with DMSO, 10 and 100 nM MBG or ouabain and incubated with the array membrane containing biotin-labeled detection antibodies. Antibody-protein complexes on the array were visualized using streptavidin-horseradish peroxidase and chemiluminescent signals were detected using x-ray film.
and 100 nM MBG or ouabain were determined by the human IL-6 TiterZyme enzyme immunometric assay (EIA) kit (Assay Designs) (51, 52). The kit uses a monoclonal antibody to human IL-6 immobilized on a microtiter plate to bind the human IL-6 in the standards or sample. CTB cells were treated with 10 and 100 nM MBG or ouabain in serum-free medium. After 24 h of treatment, media were collected from the flasks seeded with CTB cells. Cells treated with DMSO were used as controls. Some cells were pretreated for 2 h with p38 (SB 202190, Sigma) or Jnk (SP 600125, Invitrogen) inhibitors before treatment with 10 and 100 nM MBG or ouabain for 24 h. All of the samples were collected from the flasks after treatment for determination of IL-6 concentration. After a short incubation, the excess sample or standard was washed out, and an antibody to human IL-6 was added. After a short incubation, the excess antibody was washed out, and donkey anti-rabbit IgG conjugated to horseradish peroxidase was added. After a short incubation, the enzyme reaction was stopped, and the color generated was read at 450 nm.

**Statistical Analysis**—Data are presented as mean ± S.E. Data from MBG-treated and/or ouabain-treated groups were compared with vehicle (DMSO)-treated groups, and statistical comparison for multiple determinations was performed using a one-way analysis of variance with Tukey’s post hoc t test. A p value of less than 0.05 was considered significant.

**RESULTS**

**MBG and Ouabain Increased Phosphorylation of Jnk, p38, and Src**—Both 10 and 100 nM MBG and ouabain caused a significant increase in the ratio of phosphorylated Jnk to total Jnk in CTB cells compared with controls (50, 48, and 45% increase at 10, 30, and 60 min, respectively, after MBG treatment and 38, 40, and 40% increase at 10, 30, and 60 min, respectively, after ouabain treatment; p < 0.001 for each) (Fig. 1, A and B). Similarly, both 10 and 100 nM MBG and ouabain significantly increased the ratio of phosphorylated p38 to total p38 in CTB...
cells compared with controls (62, 58, and 60% increase at 10, 30, and 60 min, respectively, after MBG treatment and 57, 58, and 60% increase at 10, 30, and 60 min, respectively, after ouabain treatment; \( p < 0.001 \) for each) (Fig. 2, A and B). Both 10 and 100 nM MBG and ouabain significantly increased the ratio of phosphorylated Src to total Src in CTB cells compared with controls (43, 42, and 45% increase at 10, 30, and 60 min, respectively, after MBG treatment and 50, 48, and 52% increase at 10, 30, and 60 min, respectively, after ouabain treatment; \( p < 0.001 \) for each) (Fig. 3, A and B). Ratios of phosphoprotein to total protein at 2- and 4-h time points were not statistically different compared with 1 h for all measures (data not shown). MBG and ouabain had no effect on total Jnk, p38, and Src expression demonstrating that the changes in the ratio were because of increased phosphorylation.

**MBG- and Ouabain-activated CTB Cell Apoptosis**—Apoptosis was evaluated by caspase 9 and caspase 3/7 activation and annexin-V staining. As shown in Fig. 4, A and B, 10 nM MBG and ouabain significantly activated caspase 9 activity in a time-dependent manner in CTB cells (1.75-, 2.0-, and 2.5-fold activation at 2, 4, and 6 h, respectively, after MBG treatment and 1.5-, 2.5-, and 3.0-fold activation at 2, 4, and 6 h, respectively, after ouabain treatment). Caspase 9 was significantly activated in MBG-treated and ouabain-treated CTB cells as compared with DMSO-treated cells (\( p < 0.001 \) for basal versus 2, 4, or 6 h MBG or ouabain treatment). As shown in Fig. 5, A and B, 10 nM MBG and ouabain significantly activated caspase 3/7 activity in a time-dependent fashion in CTB cells (2.0-, 2.2-, and 2.5-fold activation at 2, 4, and 6 h, respectively, after MBG treatment and 1.5-, 1.75-, and 2.0-fold activation at 2, 4, and 6 h, respectively, after ouabain treatment). Caspase 3/7 was significantly activated in MBG-treated and ouabain-treated CTB cells as compared with DMSO-treated cells (\( p < 0.001 \) for basal versus 2, 4, or 6 h MBG treatment).

Apoptosis was also evaluated by annexin-V staining. Both 10 and 100 nM MBG or ouabain cell treatment induced positive...
annexin-V staining indicating apoptosis. This was completely reversed by inhibition of both p38 and Jnk (Fig. 6, A and B).

**MBG and Ouabain Induced IL-6 Secretion**—Both 10 and 100 nM MBG or ouabain induced the secretion of IL-6 compared with DMSO-treated CTB cells (Fig. 7, A and B). Furthermore, both 10 and 100 nM MBG and ouabain significantly increased the secretion of IL-6 compared with DMSO-treated CTB cells (2-fold increase in the concentration of IL-6 for both 10 and 100 nM MBG and ouabain; \( p < 0.001 \) for each) (Fig. 8, A and B). To test whether the increased IL-6 expression was downstream of p38 or Jnk, CTB cells were pretreated with p38 and Jnk inhibitors before treatment with DMSO, 10 and 100 nM MBG, or ouabain for 24 h. p38 inhibition significantly attenuated the IL-6 increase induced by both 10 and 100 nM MBG and ouabain \( (p < 0.001) \). However, Jnk inhibition had no effect on IL-6 secretion (Fig. 9).

**DISCUSSION**

MBG is an endogenous inhibitor of the \( \text{Na}^+ / \text{K}^+ \)-ATPase and has been shown to be an important mediator of salt sensitivity in rats \( (2, 3) \), as well as animals and humans undergoing volume expansion \( (8, 53, 54) \). It has been suggested that \( \text{Na}^+ / \text{K}^+ \)-ATPase inhibitors may contribute to the etiology of salt-sensitive- and/or volume expansion-mediated hypertension \( (4, 55) \). The postulate has been advanced that volume expansion serves as the stimulus for the elaboration of circulating inhibitors of \( \text{Na}^+ / \text{K}^+ \)-ATPase \( (55–57) \). Two classes of \( \text{Na}^+ / \text{K}^+ \)-ATPase inhibitors have been identified, the cardenolides and the bufadienolides. MBG is a representative of the latter class of compounds \( (1) \). We have proposed that a group of patients with preeclampsia have an acquired or genetic defect in sodium excretion \( (58) \). Accordingly, despite the excess secretion of these sodium transport inhibitors, these individuals are unable to excrete the excess salt and water. Instead, their vasoconstrictive/hypertensive properties become predominant.

Haddy and co-workers \( (4) \) demonstrated in a remnant rat model that injection of bufalin, a congener of MBG, causes hypertension both in normal and 70% reduced renal mass rats and Bagrov and co-workers \( (3) \) showed that MBG levels are elevated in Dahl salt-sensitive rats. Fedorova et al. \( (59) \) demonstrated that the immunoneutralization of MBG in pregnant rats with NaCl-induced hypertension, along with an antihypertensive effect, is associated with a restoration of aortic sodium pump activity. We have previously reported on a model of
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experimental preeclampsia that MBG excretion was increased when pregnant rats received 0.9% saline as their drinking water and were injected with the mineralocorticoid deoxycorticosterone acetate (7). In fact, the urinary excretion of MBG was increased before the development of hypertension, and moreover, antibodies to MBG restored blood pressure in these animals to normal levels (8). Data from a previous study from this laboratory indicate that MBG interferes with normal CTB function (17). We therefore investigated the consequences and signaling mechanisms of MBG treatment on the differentiation and invasion potential of human extravillous CTBs.

CTB cells play a key role in anchoring the placenta to the uterine wall. Hence, cell division and proliferation are requisite steps for successful placental formation (15, 16). CTB migration and invasion are highly regulated processes that directly affect pregnancy outcome (15). The endovascular invasion of CTB cells is regulated by a number of mechanisms, including the activation of multiple signaling pathways by various endogenous growth factors and/or extracellular matrix proteins. Although very little is known about MBG-induced signaling, evidence suggests that the Na⁺/K⁺-ATPase can function as a transmembrane signal-transducing complex (1, 41). Xie (60) reported that in addition to promoting ion transport, Na⁺/K⁺-ATPase acts as a signal transducer in a cell-specific manner. Allen and co-workers (61, 62) demonstrated that the cardiotoxic steroid ouabain stimulates vascular smooth muscle cell proliferation, which correlates with increased epidermal growth factor transactivation and Src phosphorylation. Moreover, binding of ouabain to the sodium pump induces the phosphorylation of various MAPKs and subsequently activates the transcription factors AP-1 (activator protein-1) and NFκB (63). In a recent study, we demonstrated that the MBG-induced impairment of CTB cell proliferation, migration, and invasion

FIGURE 7. MBG and ouabain induced IL-6 secretion. CTB cells were treated with DMSO and 10 and 100 nm MBG (A) or ouabain (B) for 24 h. The medium were subjected to a cytokine antibody array assay. Both 10 and 100 nm MBG or ouabain induced the secretion of IL-6 (indicated by arrows) compared with DMSO-treated CTB cells.

FIGURE 8. MBG and ouabain induced IL-6 secretion. CTB cells were treated with DMSO and 10 and 100 nm MBG (A) or ouabain (B) for 24 h. The media were subjected to measurements of IL-6 by EIA. Both 10 and 100 nm MBG and ouabain significantly increased the secretion of IL-6 compared with DMSO-treated CTB cells (2-fold increase in the concentration of IL-6 for both 10 and 100 nm MBG and ouabain; *, p < 0.001 for each). Results are presented as the mean ± S.E. (n = 5, eight replicates each).

FIGURE 9. MBG- and ouabain-induced IL-6 secretion was attenuated by p38 inhibition. CTB cells were pretreated with p38 (A) and Jnk (B) inhibitors before treatment with DMSO and 10 and 100 nm MBG or ouabain for 24 h. The media were subjected to measurements of IL-6 by EIA. p38 inhibition significantly attenuated the IL-6 increase induced by both 10 and 100 nm MBG and ouabain (*, p < 0.001); however, Jnk inhibition had no effect on IL-6 secretion. Results are presented as the mean ± S.E. (n = 5, eight replicates each).
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was associated with decreased ERK1/2 activity most likely mediated via the inhibition of Na⁺/K⁺-ATPase (18). However, other cellular mechanisms responsible for the MBG-induced decrease in CTB function remain unknown. Berra et al. (64) demonstrated that inhibition of basal ERK activity is sufficient to trigger apoptosis and p38 activation with no changes in Jnk phosphorylation. Chuang et al. (65) showed that CdCl₂ activated Jnk and p38 signaling with a concomitant decrease in ERK signaling. The stimulated Jnk and p38 pathways are known to regulate cell proliferation, invasion, survival, migration, growth arrest, and apoptosis (41, 20–24). To determine whether MBG-induced apoptosis might contribute to CTB dysfunction, we examined the effects of MBG on Jnk, p38, and Src activity in CTB cells. We found that MBG significantly increased the phosphorylation of Jnk, p38, and Src in CTB cells but had no effect on total expression (Fig. 1A, Fig. 2A, and Fig. 3A, respectively). Taken together with the data from our previous study (18), we conclude that MBG decreases the ERK1/2 pathway and activates the Jnk and p38 pathways. All of these actions may therefore play a role in contributing to CTB dysfunction.

In our study, we also found that MBG induced CTB apoptosis, which was demonstrated by caspase 9 and caspase 3/7 activation (Fig. 4A and Fig. 5A) and also by positive annexin-V staining (Fig. 6A). This was prevented by p38 and Jnk inhibition prior to MBG treatment (Fig. 6A). Chuang et al. (65) observed a similar type of signaling in CL13 human lung adenocarcinoma cells by cadmium. Cell death by apoptosis plays a pivotal role in the regulation of various physiologic or pathologic conditions. Apoptosis pathways may be initiated through different entry sites, such as cell receptors or mitochondria resulting in activation of effector caspases (66). Apoptotic signaling can be initiated in different cellular compartments, including the nucleus, cytosolic elements, or plasma membrane (67, 68). The damage response to an activator also involves the activation of the p38 or Jnk signaling pathways (68). These signaling cascades eventually lead to a common effector phase of apoptosis characterized by activation of caspases (67). Numerous studies suggest that caspase activation and annexin-V positive staining are valid indicators of apoptosis (32, 33, 66–68). Our study demonstrated that MBG induces apoptosis of CTB cells and suggests that Jnk and p38 cooperatively participate in the apoptosis of CTB cells induced by MBG.

Additionally, we found that MBG increases IL-6 expression (Fig. 7A and Fig. 8A). Oritani et al. (39) demonstrated that IL-6 induces apoptosis by down-regulating anti-apoptotic factors and stimulating the pro-apoptotic factor BCL-XL in 1A 9-M cells. We also found that p38 inhibition, but not Jnk inhibition, significantly attenuated the secretion of IL-6 by CTB cells in response to MBG (Fig. 9). The findings are similar to those in B9 hybridoma cells in which one of the major signal transduction pathways leading to increased IL-6 expression involves p38 but not Jnk (38). Additionally, Thobe et al. (40) suggested that the Src family of kinases regulates p38-mediated IL-6 production following hypoxia in Kupffer cells, which coincides with our observations (Figs. 3, 8, and 9).

In this study, ouabain, another Na⁺/K⁺-ATPase inhibitor, had strikingly similar effects to those of MBG on the activation of Jnk, p38, and Src phosphorylation (Fig. 1B, Fig. 2B, and Fig. 3B, respectively) as well as on apoptosis (Fig. 4B, Fig. 5B, and Fig. 6B). Ouabain had similar effects to MBG on IL-6 secretion in CTB cells that were also attenuated by p38 inhibition but not Jnk inhibition (Fig. 9). Contrary to our findings, Akimova et al. (69) demonstrated in cultured renal cells (C7-MDCK) that ouabain induced cell death independent of Na⁺/K⁺-ATPase signaling, although MBG did not have any effect. These results suggest that the effects of various cardenolides may be cell-specific as well as Na⁺/K⁺-ATPase-dependent and -independent. However, this matter will require further study. MBG- and ouabain-induced activation of Jnk, p38, and Src, activation of caspase 9 and 3/7, positive annexin-V staining, and increased secretion of IL-6 in CTB cells suggest that these cardioactive steroids increase apoptotic signaling and lead to apoptosis in CTB cells (Fig. 10). The prevention of MBG- and ouabain-induced apoptosis by p38 and Jnk inhibition and MBG- and ouabain-induced IL-6 secretion by p38 inhibition support this notion.

In summary, our data demonstrate that MBG has deleterious consequences on human CTB cell function (17, 18) and support a role for MBG in the abnormal placentation and thus altered vascular function characteristic of preeclampsia. The MBG-induced apoptosis of CTB cells is associated with the potentiation of p38 and Jnk activity and with the increased secretion of IL-6. Accordingly, the therapeutic targeting of the MBG signaling pathway may provide a new treatment paradigm for preeclampsia.

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REFERENCES
1. Schoner, W. (2002) Eur. J. Biochem. 269, 2440–2448
2. Bagrov, A. Y., and Fedorova, O. V. (1998) J. Hypertens. 16, 1953–1958
