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Magnolol stimulates steroidogenesis in rat adrenal cells

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1 This study investigated the effect of magnolol, a compound purified from *Magnolia officinalis*, on glucocorticoid production by primary adrenal cell culture.

2 Magnolol increased corticosterone secretion in a dose-dependent manner, this effect being maximal at 40 μM. A similar effect was seen in a minced adrenal gland system.

3 In magnolol-treated cells, the number and total area of cytoplasmic lipid droplets were reduced, suggesting a high utilization rate of cholesterol esters stored in lipid droplets. In control cells, the capsule of the lipid droplet was clearly delineated by immunostaining with antibody A2, whereas capsular staining was discontinuous or undetectable following magnolol treatment. The percentage of decapsulated cells increased significantly from 20% in the control group to 80% in the magnolol-treated group.

4 Magnolol-induced steroidogenesis was not mediated either *via* the traditional ACTH-cyclic AMP-protein kinase A pathway or by protein kinase C, since the intracellular cyclic AMP level did not change and inhibition of protein kinase A or C did not block the action of magnolol. Furthermore, calcium/calmodulin-dependent protein kinase II was not involved in magnolol-induced steroidogenesis.

5 The stimulatory effect of magnolol on steroidogenesis apparently requires new protein synthesis, since cycloheximide inhibited magnolol-induced corticosterone production by 50%.

6 Although other studies have shown that high concentrations of magnolol inhibit acyl-CoA: cholesterol acyltransferase and 11β-hydroxysteroid dehydrogenase in a cell-free system, our data show that, in adrenal cell cultures, low concentrations of magnolol have a stimulatory effect on steroidogenesis, and the glucocorticoid produced may explain the effective control of asthma by *Magnolia officinalis*.

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Abbreviations: DMEM, Dulbecco's modified Eagle's medium; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol 12-myristate-13 acetate

Introduction

Magnolia officinalis, a Chinese medicinal herb, has long been used for the relief of fever, headache, anxiety, diarrhoea, stroke, and asthma. Magnolol, one of the compounds isolated from *Magnolia officinalis* (Lo *et al*., 1994), acts on the central nervous system (Watanabe *et al*., 1983), inhibits platelet coagulation (Teng *et al*., 1988) and lymphocyte proliferation (Hirano *et al*., 1991), and is an anti-inflammatory agent (Wang *et al*., 1992) and smooth muscle relaxant (Teng *et al*., 1990).

Magnolol also suppresses the conversion of cortisol to cortisone in a dose-dependent manner by targeting 11β-hydroxysteroid dehydrogenase in the liver homogenate assay system (Homma *et al*., 1994). In addition, it is involved in lipid metabolism by inhibiting acyl-CoA: cholesterol acyltransferase, which catalyzes cholesterol esterification and facilitates the accumulation of intracellular cholesterol ester and has therefore been suggested as a cholesterol lowering agent for the treatment of hypercholesterolaemia and atherosclerosis (Kwon *et al*., 1997). Recently magnolol has been shown to prevent neointimal hyperplasia effectively in hypercholesterolaemic rabbits, characterized by the accumulation of foam cells (lipid droplet-containing macrophages) in the subendothelial space (Chen *et al*., personal communication). We were therefore interested in understanding the underlying mechanism of action of magnolol in macrophage lipid metabolism and determining whether it stimulates lipolysis or blocks the accumulation of cholesterol ester in lipid droplets.

Cultured adrenal cells contain many lipid droplets and the lipolytic end product (corticosterone) can be readily assayed. This bioassay system therefore is a good model in which to study the effect of magnolol on adrenal cell lipolysis. Although lipolysis can be estimated by the amount of corticosterone secreted by the cells, another criterion is the decapsulation of the lipid droplet capsule in these cells. Electron microscopic study shows that lipid droplets in bovine fasciculata cells are surrounded by a layer of an electron-dense substance, known as the 'capsule' (Almahbobi *et al*., 1992). In adrenal cells, this lipid droplet capsule can be demonstrated by immunostaining with a monoclonal antibody A2, which recognizes a 160 kDa protein on the lipid droplet surface (Wang & Fong, 1995; Fong & Wang, 1997). The detachment of the capsule from the lipid droplet surface is known as 'decapsulation'; it is characterized by discontinuous or weak capsule staining with antibody A2 and is promoted by activation of PKA or inhibition of PKC (Fong & Wang, 1997). The same signal pathways (PKA activation and PKC inhibition) that mediate lipid droplet decapsulation also control steroidogenesis in adrenal cells (Shima *et al*.,

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1971; Widmaier & Hall, 1985; Simpson & Waterman, 1988; Reyland, 1993). Therefore, antibody A2 can be used as a convenient marker to study capsule structure. In adipocytes and steroidogenic cells, perilipin and adipophilin are also present on the lipid droplet surface (Greenberg *et al*., 1991; Servetnick *et al*., 1995; Londos *et al*., 1996; Brasaemal *et al*., 1997; Heid *et al*., 1998).

In the present study, we examined the effect of magnolol on adrenal cell corticosterone secretion and lipid droplet capsular morphology, revealed by immunostaining with antibody A2. In order to elucidate the signalling pathway involved in the action of magnolol, the effect of co-treatment with magnolol and specific signalling pathway inhibitors was tested.

Methods

Cell culture

Adult female Wistar rats at the age of 8–10 weeks were purchased from the animal centre at National Taiwan University. The animals were killed with an overdose of 7% chloral hydrate (6 ml kg^{-1}) by i.p. injection. Adrenal glands were isolated, minced, and digested for 30 min at 37°C with 1 mg ml^{-1} of type I collagenase in DMEM (Gibco, Long Island, U.S.A.). After mechanical dispersion with a glass pipette, the dissociated cells were collected by centrifugation ($1000 \times g$) and cultured in a 1 : 1 v v^{-1} mixture of F-12 medium and DMEM, supplemented with 1.2 g l^{-1} of $NaHCO_3$, 5% horse serum, 2.5% foetal calf serum, and 1% penicillin and streptomycin. The cells were then cultured at 37°C in an atmosphere of 95% air and 5% CO_2 in 24-well plates (for corticosterone assay) or on grid-coverslips (Bellco Glass Inc., Vineland, NJ, U.S.A.) (for morphology studies).

Corticosterone radioimmunoassay

Day 3–4 adrenal cultures were used in the following experiments. After drug treatment, 5 μl of culture medium was diluted 1 : 100 with assay buffer (0.05 M Tris-HCl, pH 8.0, 0.1 M NaCl, 0.1% NaN_3, and 0.1% BSA), then mixed at room temperature with 500 μl of a 1 : 10 dilution of rabbit anti-corticosterone antiserum (C-8784; Sigma). After 30 min, 100 μl of H^3-labelled corticosterone (TRK 406; Amersham, Buckinghamshire, U.K.) was added to each assay tube and the mixture incubated at 37°C for 1 h and at 4°C for a further hour. Free corticosterone was adsorbed by incubation for 10 min at 4°C with 200 μl of dextran-coated charcoal (0.5% dextran and 1.25% charcoal in assay buffer) and the bound corticosterone separated by centrifugation at $13{,}000 \times g$ for 10 min at 4°C. 0.7 ml of the supernatant was then mixed with 3 ml of scintillation solution (Ecoscient H) before counting in a β-counter (LS 600IC: Beckman, Fullerton, CA, U.S.A.) for 1 min. A standard curve was prepared using a corticosterone standard (Sigma). The protein concentration of the cell pellets was determined by colorimetric assay at 595 nm using the Biorad protein assay kit (Biorad), and corticosterone production expressed as pg mg^{-1} protein. Each experiment was performed in triplicate, and the data represent the mean ± s.d. $P < 0.05$ was considered significant using Student's *t*-test.

Drug treatment

Magnolol is an amphipathic molecule composed of two phenol rings with one polar hydroxyl and one nonpolar hydrocarbon domains on each phenol ring (Teng *et al*., 1988). This unique property allows magnolol to freely permeate through the cell membrane and enter the cytoplasm. The preparation purchased from Pharmaceutical industry technology and development centre in Taiwan was characterized by two types of HPLC columns (Cosmosil 5C 18-AR and Nucleosil 10 μm NH_2) and purified to >99%. Magnolol (30 mM in dimethyl sulphoxide) was added to the culture medium at final concentrations of 10, 20, 30 and 40 μM for different time intervals. A PKA inhibitor [H-8 (RBI)], PKC inhibitor [(calphostin C (RBI)], or PKC activator [PMA (RBI)] was added either alone or together with magnolol (30 μM) for 3 or 6 h, then the culture medium was collected and assayed for corticosterone, while the cells were assayed for protein. The effect of magnolol on corticosterone production in adrenal glands was also tested *in situ*. Minced adrenal glands were incubated with 30 μM magnolol in culture medium for 3 h at 37°C in an CO_2 incubator, then the culture medium was collected for corticosterone assay and the tissue fragments homogenized and assayed for protein.

Cyclic AMP assay

Adrenal cells were treated for 30 min with magnolol (30 μM) and 3-isobutyl-1-methylxanthine (500 μM; Sigma). The culture medium was then aspirated off and the cells washed with PBS, then treated overnight with 0.5 ml of 0.01 N HCl to extract the cyclic AMP, which was measured using the Amersham (Arlington Heights, IL, U.S.A.) [H^3]-cyclic AMP assay system. A standard curve for cyclic AMP was prepared using a cyclic AMP kit from Amersham.

Immunofluorescence microscopy and morphometry of lipid droplets

Adrenal cells on coverglasses were fixed for 5 min in PBS containing 0.15% glutaraldehyde and 0.1% Triton X-100, then treated for 30 min at room temperature with $NaBH_4$ (1 mg ml^{-1} in PBS). After PBS washes, the cells were incubated for 2 h at 37°C with diluted monoclonal antibody A2, which binds specifically to the lipid droplet capsule (Wang & Fong, 1995). After a 15 min wash in PBS, the cells were incubated for 1 h at 37°C with FITC-conjugated goat anti-mouse IgG, washed with PBS, and mounted for examination with a Leica fluorescence microscope (Polyvar, Leica, Vienna). To estimate the percentage of decapsulated cells, three samples from each treatment group were examined. A decapsulated cell was defined as one in which the majority of lipid droplets were not immunostained with antibody A2 on the lipid droplet surface. To determine the change in total surface area of lipid droplets in a cell after magnolol treatment, we analysed time-course recorded phase images using the program Image Pro-plus, version 3.0 (Media Cybernetics, U.S.A.).

Results

Effect of magnonol on corticosterone production

The control group of adrenal cells showed a basal level of corticosterone secretion (Figure 1, vehicle only). Magnolol stimulated the release of corticosterone in a dose-dependent manner (Figure 1), increasing significantly between 3 and 24 h after magnolol application. Since high concentrations

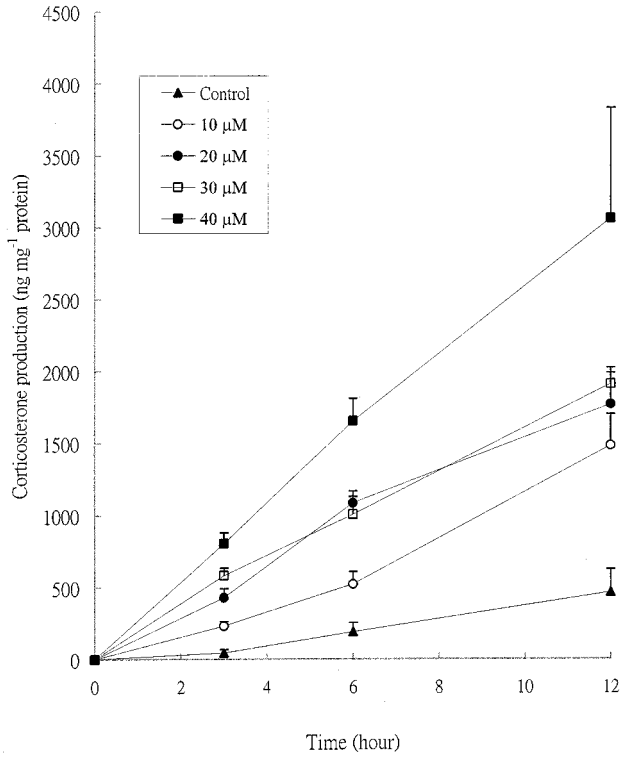

Figure 1 Time-course and dose response effect of magnolol on corticosterone production. 10, 20, 30 and 40 indicate the concentrations of magnolol in μM. Using 30 μM magnonol, a significant increase was seen at 3 h and this effect lasted for 12 h.

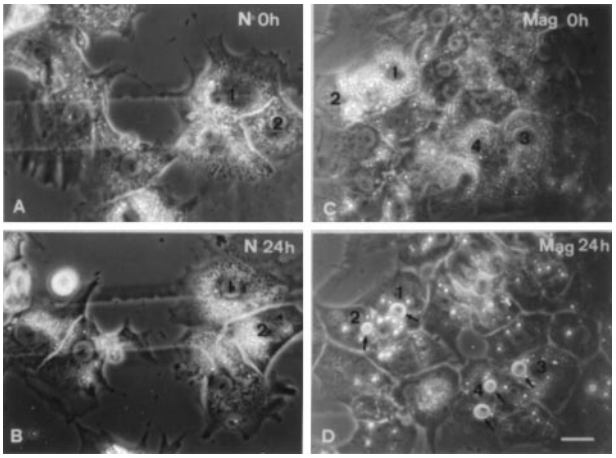

Figure 2 Effect of magnolol on adrenal cell morphology. Phase images of control (A,B) and magnolol-treated cells (C,D) recorded at 0 h (A,C) and 24 h (B,D). In control cells, the same number of lipid droplets were seen at 0 h (A) and 24 h (B). Magnolol (30 μM) reduces the number of small lipid droplets in cells 1–4 (D) and induces the formation of large lipid droplets (arrows in D). Scale bar = 10 μm.

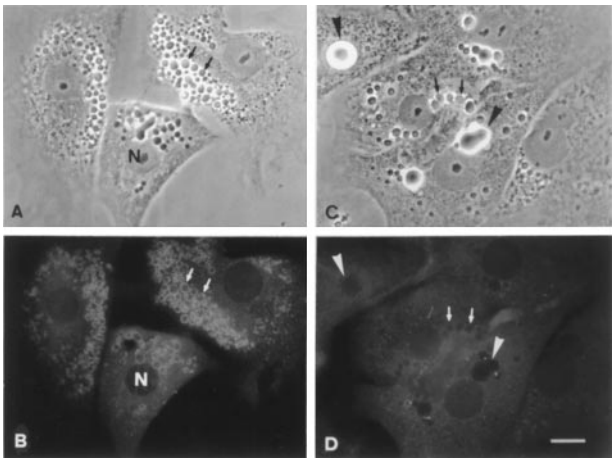

Figure 3 Effect of magnolol on capsular morphology of lipid droplets. Control (A,B) and magnolol (30 μM)-treated cells (C,D) immunostained with A2 antibody. A,C: phase images; B,D: immunofluorescence images. In control cells, A2 antibody labels a complete capsule on each lipid droplet (arrows in B). After magnolol treatment, A2 staining is not seen on the surface of small (arrows in D) and large lipid droplets (arrowheads in D). Scale bar = 20 μm.

(>40 μM) and prolonged treatment resulted in cell destruction and detachment from the substratum, we routinely used 30 μM as the working concentration in the following experiments. The potential of magnolol to stimulate corticosterone release was also confirmed *in situ*, when adrenal tissue fragments were incubated for 3 h with magnolol, the corticosterone concentration in the culture medium increased to $156 \pm 28\%$ of the control value.

Effect of magnolol on lipid droplet morphology

Control adrenal cells contained many small lipid droplets in the cytoplasm (Figure 2A); after 24 h of culture, the relative position of the lipid droplets changed (Figure 2B) and the total area of the lipid droplets decreased to $89 \pm 30\%$ as measured by quantitative analysis of the phase images. In cells treated for 24 h with magnolol, the number of small lipid droplets decreased significantly and a few large lipid droplets appeared (arrowheads in Figure 2D), and the total area of the lipid droplets fell to $57 \pm 21\%$ of the control levels. In addition to the size and number of lipid droplets, a distribution change in the capsular morphology of lipid droplets was also noted. Normal cells contained an intact capsule, seen as a complete ring on the surface of lipid droplets of varying sizes (arrowheads in Figure 3A,B). The majority of lipid droplets showed no capsular staining (arrowheads in Figure 3C,D) or only retained an incomplete capsule, shown as discontinuous fluorescent dots on the surface of the lipid droplets. These cells were defined as 'decapsulated cells'. Quantitative analysis showed that the ratio of decapsulated cells increased from 19.46% in the control group to 77.2% in the magnolol-treated group (Figure 4).

Mechanism of magnolol action

Since PKA activation is accompanied by an increase in the level of intracellular cyclic AMP, in order to determine whether magnolol-induced steroidogenesis involved PKA activation, we measured cyclic AMP levels in control and magnolol-stimulated adrenal cells. The results showed that magnolol treatment did not result in an increase in intracellular cyclic AMP levels, whereas an ACTH-stimulated group showed a 5 fold increase in cyclic AMP levels. A PKA inhibitor, H-8, was then tested for effects on magnolol-induced steroidogenesis. Treatment with H-8 alone for 3 or 6 h had no effect on the basal level of steroidogenesis. Combination of H-8 and magnolol did not affect corticosterone production induced by magnolol alone. Furthermore, 80% of magnolol-treated adrenal cells were decapsulated compared to 20% in the control group, whereas magnolol/H-8 cotreatment resulted in 77% of cells being decapsulated,

indicating that H-8 did not inhibit the action of magnolol on capsule detachment. The above experiments show that PKA activation is not involved in magnolol-induced steroidogenesis. Although the action of magnolol is PKA-independent, cotreatment with ACTH (PKA-dependent) did not have additive effect on magnolol-induced steroidogenesis (unpublished data).

The principal PKC isoforms in rat adrenal cortex is the α form with minor expression of δ, γ and ζ (Pelosin *et al*., 1991; Wetsel *et al*., 1992).To determine whether PKC was involved in magnolol action, the effects of PMA, a PKC activator, and calphostin C, a PKC inhibitor, on magnolol-induced steroidogenesis were examined. PMA activates PKC (high affinity receptor for PMA) by direct binding to PKC, and calphostic C interacts with the regulatory domains of conventional and novel PKC isoforms (Kobayashi *et al*., 1989; Ron & Kazanietz, 1999). Both drugs directly act on PKC bypassing the phospholipase-diacylglycerol pathway. Treatment with magnolol, PMA, or calphostin C alone increased corticosteroid production within 3 or 6 h of treatment (Figure 5). Cotreatment with magnolol and PMA or calphostin C resulted in an additive increase in corticosteroid production, suggesting that the signalling pathway involved in magnolol stimulation is PKC-independent.

In bovine adrenal fasciculata cells (Nishikawa *et al*., 1997) and in human adrenocortical H295R cells (Pezzi *et al*., 1996), inhibition of calcium/calmodulin-dependent protein kinase II by the specific inhibitor, KN-93, blocks the activation of steroidogenic acute regulatory protein (StAR), which regulates the movement of cholesterol from the outer to the inner mitochondrial membranes. In the present study, we found that KN-62 did not inhibit magnolol-induced corticosterone production (Figure 5).

To investigate whether magnolol-induced steroidogenesis requires new protein synthesis, cycloheximide, a protein synthesis blocker, was added at the same time as magnolol, resulting in a 50% reduction in magnolol-induced corticosterone production at 3 or 6 h (Figure 6), suggesting that magnolol stimulates new synthesis of proteins required in the magnolol-induced steroidogenesis pathway. Morphological observations supported the above data. Complete capsule staining was seen in cycloheximide-treated cells (Figure 7A). Magnolol induced detachment of the capsule from lipid droplet surface (Figure 7B), however, cotreatment of magnolol with cycloheximide partially inhibited the detachment of the capsule (Figure 7C).

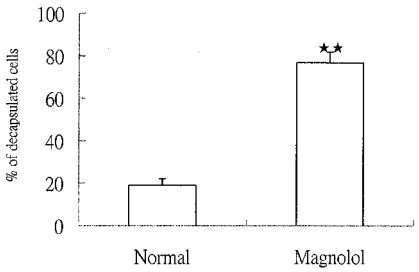

Figure 4 Effect of magnolol on capsule detachment from adrenal cells. For both control and magnolol-treated (30 μM, 5 h) cells, three immunostained coverslips were examined and 50 cells on each coverslip chosen and classified as intact or decapsulated cells. Values represent the percentage of decapsulated cells (mean$\pm$s.d.). (**, $P<0.01$ vs normal).

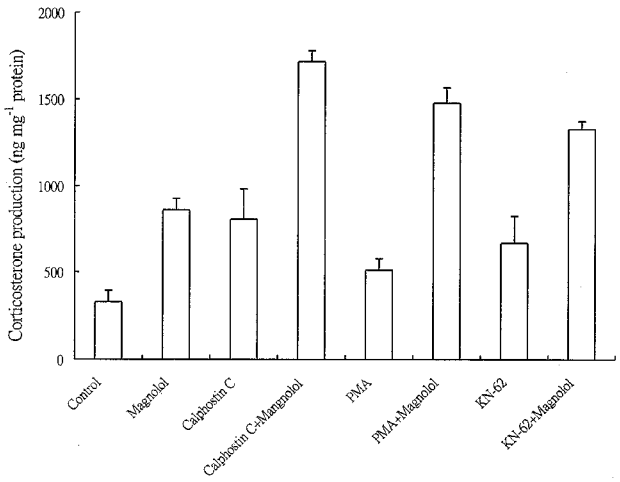

Figure 5 Effect of PMA, calphostin C and KN-62 on magnolol-induced steroidogenesis. Cotreatment with PMA (100 nM), calphostin C (500 nM) or KN-62 (2 μM) resulted in an additive effect on magnolol-induced steroidogenesis.

Discussion

The present study first demonstrated that magnolol, a compound purified from *Magnolia officinalis*, has a direct stimulatory effect on steroidogenesis in rat adrenal cells. The *in vitro* stimulatory effect of magnolol on cultured adrenal cells was confirmed *in situ*; direct incubation of magnolol with adrenal gland fragments induced an increase in corticosterone secretion. This traditional Chinese

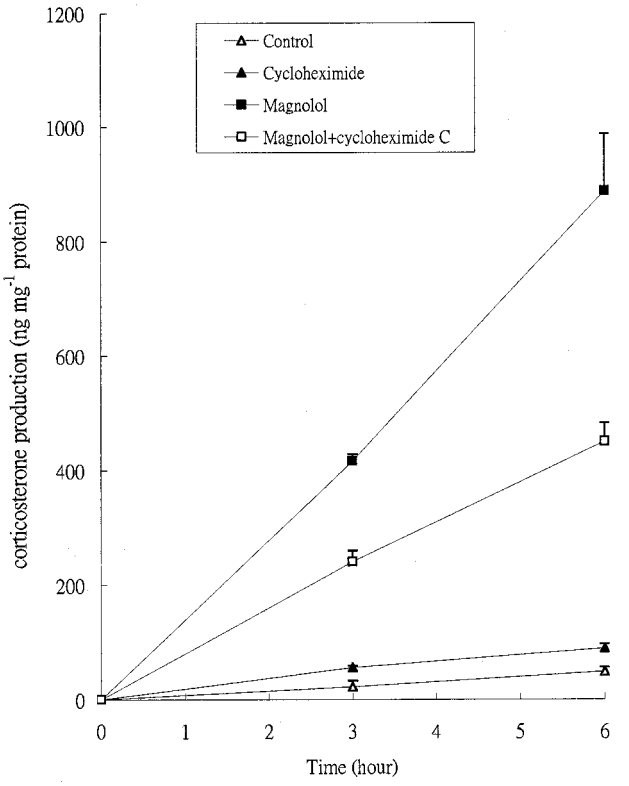

Figure 6 Effect of cycloheximide on magnolol-induced steroidogenesis. Adrenal cells were treated with 30 μM magnolol in the presence or absence of 2 μM cycloheximide for 3 or 6 h and the culture medium collected for corticosterone measurement. Cotreatment with cycloheximide resulted in about 50% of the corticosterone production induced by magnolol alone.

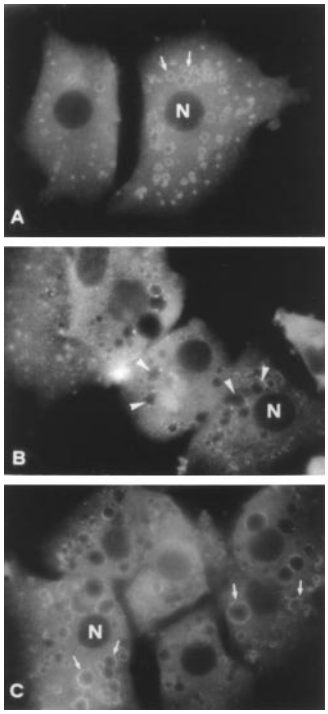

Figure 7 Effect of cycloheximide on magnolol-induced decapsulation of lipid droplets in adrenal cells. Cells were treated for 3 h with 2 μM cycloheximide (A), 30 μM magnolol (B), or 2 μM cycloheximide plus 30 μM magnolol (C) before immunostaining with antibody A2. The lipid droplet capsule was intact in cells treated with cycloheximide alone (arrows, A) and partially retained in cells cotreated with magnolol and cycloheximide (arrows, C). Capsular staining of lipid droplets was no longer detected after magnolol treatment (arrowheads in B, control see Figure 2B). Scale bar = 10 μm.

medicine has been used to improve the symptoms of asthma. Glucocorticoids are effective anti-asthma agents and may directly modulate airway smooth muscle contraction, augment smooth muscle relaxation and inhibit smooth muscle proliferation, as well as preventing the release and/or activity of several cytokines and other pro-inflammatory mediators (Hirst & Lee, 1998). The present study suggests that some of these effects of *Magnolia officinalis* may be due to its stimulatory effect on corticosterone secretion by the adrenal gland, which, in turn, mimics the action of exogenous glucocorticoids in the treatment of asthma.

Evidence for the effects of magnolol on steroidogenesis came not only from the biochemical assay of corticosterone, but also from morphological analyses of intracellular lipid droplets. Cytoplasmic lipid droplets decreased in number and became fused into a few large lipid droplets. The fusion of lipid droplets may be due to the loss of the lipid droplet capsule, which acts as a barrier preventing lipid droplets from fusing with one another. The loss of the capsule, shown by antibody A2 staining of those lipid droplets remaining after magnolol treatment, may facilitate lipolysis by translocation of cytoplasmic cholesterol ester hydralase to the surface of the lipid droplet. The percentage of adrenal cells containing decapsulated lipid droplets increased significantly from 20 to 80% after magnolol treatment, suggesting that magnolol enhances the mobilization of cholesterol from the lipid droplet storage site.

It is interesting to note that magnolol treatment reduces the number of intracellular stored lipid droplets. This compound inhibits rat liver acyl-CoA:cholesterol acyltransferase with an IC_{50} value of 86 μM (Kwon *et al*., 1997) and thus inhibits the formation of intracellular cholesterol esters. Failure of cholesterol ester formation may result in relatively increased levels of free cholesterol, which may provide a faster precursor pool for corticosterone synthesis and account for the increased production of corticosterone induced by magnolol. One possible explanation for the magnolol-induced reduction in the number of lipid droplets is that it blocks the accumulation of lipid droplets. Magnolol also inhibits the conversion of cortisol to cortisone in liver homogenates by inhibiting 11β-hydroxysteroid dehydrogenase with an IC_{50} of 180 μM (Homma *et al*., 1994). Both the above studies used the liver homogenate assay system and high concentrations of magnolol. We have found 30 μM magnolol to be the optimal working concentration, far lower than 86 or 180 μM, the IC_{50} values for the enzymes acyl-CoA: cholesterol acyltransferase and 11β-hydroxysteroid dehydrogenase, respectively. In this study, concentrations of magnolol greater than 40 μM resulted in apparent deterioration of adrenal cells, possibly due to the strong anti-oxidant activity of magnolol. Inhibition of acyl-CoA: cholesterol acyltransferase and 11β-hydroxysteroid dehydrogenase may occur at high magnolol concentrations and in the lysed cell system, while low magnolol concentrations may have a completely different effect on steroidogenesis.

ACTH-induced steroidogenesis in adrenal cells involves adenyl cyclase activation, increases in cyclic AMP levels, and PKA activation (Shima *et al*., 1971; Simpson & Waterman, 1988). We therefore examined whether the action of magnolol is mediated *via* the PKA pathway. Firstly, magnolol did not increase intracellular cyclic AMP levels. In addition, addition of PKA inhibitor did not affect magnolol-induced corticosterone secretion and lipid droplet decapsulation. These results show that the action of magnolol does not depend on PKA signalling pathway.

In determining whether PKC was involved in magnolol-induced steroidogenesis, we found paradox cells that both PMA and calphostin C stimulated adrenal steroidogenesis, though in a different degree. There are also conflicting data in the literature. Thus, in mouse adrenal Y-1 cells, PKC activation suppresses steroidogenesis (Reyland, 1993; Reyland *et al*., 1998) while in bovine fasciculata cells, PKC activation increases corticosteroid production (Culty *et al*., 1984; Hartigan *et al*., 1995). Since both PMA and calphostin C showed additive effects on magnolol-induced steroidogenesis, it appears that the action of magnolol is PKC-independent. In neutrophils, magnolol reduces PMA-induced aggregation by inhibiting PKC (Wang *et al*., 1998). Thus, the effect of magnolol on PKC depends on the cell type studied. Moreover, magnolol treatment did not increase the intracellular Ca^{2+} level of adrenal cells (unpublished data). Our observation is consistent with that magnolol inhibits the intracellular mobilization of Ca^{2+} in platelets and vascular smooth muscle cells (Teng *et al*., 1988; 1990).Thus, it is unlikely that magnolol action is mediated *via* phospholipase-diacylglycerol pathway.

Previously, we reported that a water-soluble extract of *Cordyceps sinesis* can directly stimulate steroidogenesis in adrenal cells in a PKA-independent, but PKC-dependent, fashion (Wang *et al*., 1998). Both *Magnolia officials* and *Cordyceps sinesis* are Chinese herbal medicines used in the treatment of asthma (Feng *et al*., 1987; Nagano *et al*., 1988; Chang *et al*., 1997), although these two drugs operate by different mechanisms. We provide here evidence that one mechanism involved in the anti-asthma effect of these two medicines is an effect on adrenal steroidogenesis, since

glucocorticoids are effective anti-asthma drugs (Hirst & Lee, 1998).

Since calcium/calmodulin-dependent protein kinase is involved in ACTH activation of StAR expression and enhances steroid production (Nishikawa *et al*., 1997), we used the inhibitor, KN-62, to examine whether calcium/calmodulin-dependent protein kinase was involved in the action of magnolol and found that it had no effect on magnolol-induced steroidogenesis. This again confirms that the mechanism of magnolol does not involve the ACTH-calcium/calmodulin-dependent protein kinase pathway. Magnolol is highly cell-permeable due to its lipophilic nature and reactive with membrane components, it remains to be determined whether it has a direct action on steroidogenic organelles, such as the sER, peroxisomes, lysosomes, and mitochondria. However, the possibility that magnolol may act *via* intracellular receptors to regulate steroidogenesis cannot be excluded.

It is interesting to note that cycloheximide blocked the increase in steroidogenesis and decapsulation of lipid droplets which were normally induced by magnolol. Cycloheximide is known to block ACTH-stimulated steroidogenesis in normal adrenal (Garren *et al*., 1965) by inhibiting the synthesis of a labile protein for cholesterol transport to cytochrome P-450$_{scc}$ (Crivello & Jefcoate, 1978; DiBartolomeis & Jefcoate, 1984). Later, this labile protein is characterized to be StAR (steroidogenic acute regulatory protein), which is activated by protein kinase A phosphorylation, and is required for the transport of cholesterol from the outer to the inner mitochondria membrane (Strauss *et al*., 1999). Therefore, cycloheximide treatment alone completely blocked steroidogenesis, which could be operated by the above mechanism. However, magnolol was able to stimulate corticosterone synthesis even under protein inhibition, suggesting that pre-existing enzymes necessary for steroid synthesis were activated by magnolol. In addition, we found that the magnolol-induced detachment of 160 kDa protein from the lipid droplet surface was also impeded by cycloheximide, indicating that cycloheximide inhibited the synthesis of some proteins which are involved in the conformational modification of the 160 kDa protein to facilitate its detachment from lipid droplet surface. Therefore, the present study demonstrated that the mobilization of free cholesterol from lipid droplets was also blocked by cycloheximide.

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