Effects of α-MSH on Intracellular Calcium Concentration in Immortalized Sebocytes

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Effects of α-MSH on Intracellular Calcium Concentration in Immortalized Sebocytes

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Abstract

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Excessive sebum secretion is known as the most important of the four traditional factors in the pathogenesis of acne. The five famous sebum controlling factors are androgen/estrogen, IGF/GF/insulin, PPAR, retinoid, and melanocortin; we focused on melanocortin, a stress related peptide.

Many other studies have suggested the pathogenesis of stress induced sebum secretion, that is melanocortins, stress induced pituitary peptide hormones such as ACTH 1-17, ACTH1-10, α -MSH bind MC5R in differentiated sebocyte and it could stimulated sebocyte to secrete sebum. Recently, a study using HEK293 cells transfected with mouse MC5R showed that melanocortin peptides stimulated a rapid and transient increase in $[Ca^{2+}]_i$, and the increase in $[Ca^{2+}]_i$ was of intracellular origin. Other recent studies showed that both human primary cultured sebocytes and immortalized human sebocytes have MC1R, but MC5R was only found in differentiated human primary cultured sebocytes.

I proposed that differentiated immortalized human sebocyte could also have MC5R and the increase in $[Ca^{2+}]_i$ could be a signaling molecule to increase sebum in immortalized human sebocytes (SZ95). Our results

showed that differentiated SZ95 cells express MC5R, α -MSH increases in $[Ca^{2+}]_i$, the increase in $[Ca^{2+}]_i$ were of intracellular origin, it might be mediated by inositol triphosphate pathway, and α -MSH stimulation increase sebum.

Key Words : calcium, α-MSH, sebocyte, sebum

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I. INTRODUCTION

Classical acne pathogenesis has four key factors: excessive sebum secretion, abnormal keratinization of follicles, *propionibacterium acne* overgrowth, and perifollicular inflammation. Of the above four factors, authors have focused on excessive sebum secretion, which is an important factor in stress-related acne pathogenesis.[1] Many studies have shown that androgen/estrogen, IGF(insulin like growth factor)/GF(growth factor)/insulin, PPARγ (peroxisome proliferators-activated receptor gamma), retinoid, and melanocortin are five important sebum controlling factors.[2]

Melanocortins, the products of the proopiomelanocortin (POMC) gene, include adrenocorticotrophin hormone (ACTH), and α -, β-, and γ-melanocyte-stimulating hormone (MSH). They regulate a variety of physiological processes such as steroidogenesis in the adrenal cortex, body weight, sexual behavior, inflammation, pain, and neuronal regeneration. In

skin, melanocortins are involved mainly in modulation of pigmentation, immune responses, and secretion from exocrine glands. Melanocortin peptides elicit their functions by binding to and activating their receptors. Thus far, five melanocortin receptors (MCRs), MC1R–MC5R, have been identified. They are all seven-transmembrane G-protein coupled receptors, which stimulate cyclic adenosine monophosphate (cAMP) upon activation.[3]

Only MC1R and MC5R have been identified in human skin. MC1R is detected mainly in hair follicle epithelia, melanocytes, some periadnexal mesenchymal cells, sebaceous glands, and secretory and ductal epithelia of sweat glands.[4] It is also detected in the immortalized human sebocyte cell line SZ95.[5] MC5R is localized to sebaceous glands, eccrine glands, hair follicles, and epidermis in human skin, as well as in cultured human sebocytes. It is detected only in differentiated, lipid-laden human skin, and in cultured human sebocytes but not in basal, undifferentiated sebaceous cells.[6]

Recently, a great deal of attention has focused on calcium ion mobilization mechanisms responsible for responding to changes in the cellular redox state. calcium ion plays a role in the regulation of a diverse range of cellular functions, such as muscle contraction, secretion, synaptic plasticity, cell proliferation, and cell death.[7] Many hormones and neurotransmitters increase intracellular calcium ion concentration ($[Ca^{2+}]_i$) by mobilizing calcium ion from intracellular stores and by inducing an influx from the extracellular space.[8, 9]

Melanocortins initiate intracellular signaling by binding to and activating G-protein coupled MCRs, resulting in increased production of cAMP and protein kinase A (PKA) activity. Moreover, recent evidence indicates that stimulation of all murine melanocortin receptors with α-MSH also elevates $[Ca^{2+}]_i$.[10] In HEK 293 cells transfected with mouse MC5R, α-MSH and ACTH peptides increased the production of cAMP and also stimulated a rapid and transient increase in $[Ca^{2+}]_i$.(Fig 1)[11]

I propose that differentiated immortalized human sebocytes could also express

MC5R and that α-MSH stimulation could increase both $[Ca^{2+}]_I$ and sebum production in immortalized human sebocytes(SZ95). We also attempted to find the pathway of $[Ca^{2+}]_i$ in SZ95 sebocytes.

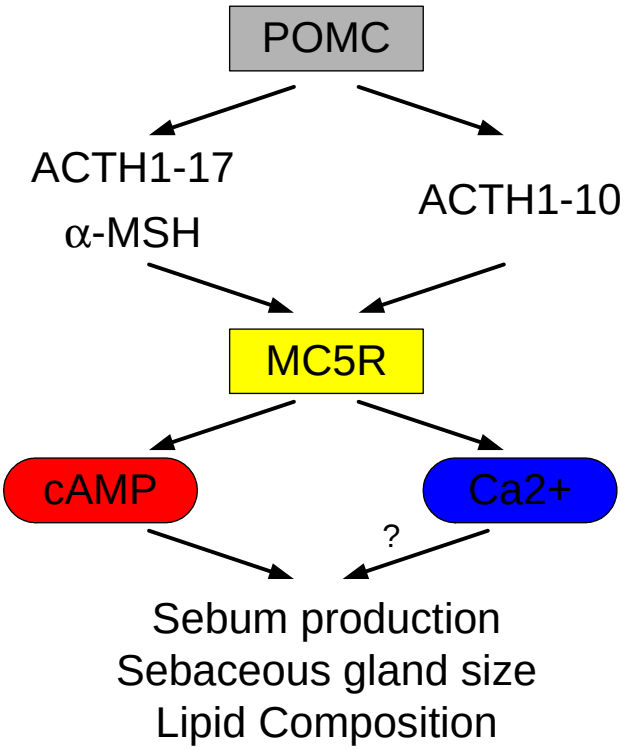


Fig 1. The effects of melanocortins on sebocytes and their effected pathways

II. Materials and Methods

1. Methods

A. Cell Culture

Immortalized human sebocyte cell line SZ95, which show the major characteristics of normal sebocytes were maintained in Dulbecco's modified Eagle medium(DMEM)/F-12 supplemented with 2 mM glutamax I, 10 ug/ml gentamicin, 50 ng/ml human epidermal growth factor(hEGF), 10% fetal bovine serum, 10 mM HEPES (Gibco BRL).

B. Measurement of $[Ca^{2+}]_i$ [12]

i. $10^5 \sim 10^6$ number of SZ95 cells were cultured in 35Φ dish for 2 days and all the culture medium was removed at 70-80 % confluency.

ii. The cell attached dish was loaded with 1 mL regular solution (5L NaCl 40.9g, HEPES 11.9g, Glucose 10g, KCl 25 mL, $MgCl_2$ 5 mL $CaCl_2$ 5 mL, pH 7.4, 310 mOsm) with Fura-2, AM 6 μL and Pluronic F-127 20% solution in DMSO 6 μL for 40 min at 37°C in the dark. In Ca^{2+}-free solutions, $CaCl^2$ was omitted, and 1 mM EGTA was added.

iii. The cells were then washed twice and rested for at least 20 min before use.

iv. The fura-2-loaded cells were mounted on the stage of an inverted microscope (Nikon, Tokyo, Japan) for imaging.

v. The cells were superfused at a constant perfusion rate with the regular solution.

vi. The excitation wavelength was alternated between 340 and 380 nm, and the emission fluorescence was monitored at 510 nm with a CCD camera using MetaFluor system (Universal Imaging Co., Downingtown, PA). Fluorescence images were obtained at 4-s intervals. Background fluorescence was subtracted from the raw signals at each excitation wavelength, and the values of $[Ca^{2+}]_i$ were calculated from Grynkiewicz equation.

C. $[Ca^{2+}]_i$ measurement after α-MSH stimulation

SZ95 sebocytes cultured in 10mM testosterone for 2 days were stimulated with 500mM of α-MSH. $[Ca^{2+}]_i$ was measured by the method described in B.

D. $[Ca^{2+}]_i$ measurement after α-MSH stimulation without extracellular calcium

SZ95 sebocytes cultured in 10mM testosterone for 2 days were stimulated with 500μM of α-MSH. $[Ca^{2+}]_i$ was measured by the method described in B with calcium-free regular solution.

E. $[Ca^{2+}]_i$ measurement after thapsigargin treatment and α-MSH stimulation

SZ95 sebocytes cultured in 10mM testosterone for 2 days were treated with thapsigargin 1μM and then stimulated with 500μM of α-MSH. $[Ca^{2+}]_i$ was measured by the method described in B.

F. $[Ca^{2+}]_i$ measurement after caffeine treatment and α-MSH stimulation

SZ95 sebocytes cultured in 10mM testosterone for 2 days were treated with caffeine 10mM and then stimulated with 500μM of α-MSH. $[Ca^{2+}]_i$ was measured by the method described in B.

G. $[Ca^{2+}]_i$ measurement after 2-aminoethoxydiphenyl borate(2-APB) treatment and α-MSH stimulation

SZ95 sebocytes cultured in 10mM testosterone for 2 days were treated with 2-APB 75μM and then stimulated with 500μM of α-MSH. $[Ca^{2+}]_i$ was measured by the method described in B.

H. $[Ca^{2+}]_i$ measurement after high concentration ryanodine treatment and α-MSH stimulation

SZ95 sebocytes cultured in 10mM testosterone for 2 days were treated with ryanodine 100μM and then stimulated with 500μM of α-MSH. $[Ca^{2+}]_i$ was measured by the method described in B.

I. $[Ca^{2+}]_i$ measurement after phospholipase C (PLC) inhibitor (u-73122), PLC control (u-73343) treatment and α-MSH stimulation

SZ95 sebocytes cultured in 10mM testosterone for 2 days were treated with u-73122 10μM and u-73343 10μM and then stimulated with 500 μM of α-MSH.

$[Ca^{2+}]_i$ was measured by the method described in B.

J. Immunohistochemical stain of MC5R in immortalized human sebocyte (SZ95) after 2 days testosterone treatment

SZ95 sebocytes were plated in wells of Lab-Tek 4-chamber slides and cultured as described above. The cells were fixed for 30 min with 10% buffered formalin (Poly Scientific R&D Corp.) and the slides were then washed three times with 1× PBS.

The nonspecific binding was blocked with DakoCytomation Protein Block Serum-Free Ready-to-Use(DakoCytomation, Carpinteria, CA, USA) for 15 min. Then, cells were incubated with the MC1R antibody (10 μg/mL) and MC5R (4 μg/mL) antibody(Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 30 min at RT, respectively. Slides were washed with PBS and incubated with a donkey anti-goat antibody conjugated with Rodamine (1:100, Santa Cruz) for 30 min at RT. After washing, the slides were mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA) and stored until use. Cells were examined and imaged with confocal microscope(Zeiss LSM 510 meta, Carl Zeiss Microscope Systems, Jena, Germany). Red fluoroscence was excited at 540 nm and emission was measured at 580 nm.

K. Nile red stain after 2 days testosterone and α-MSH treatment

SZ95 sebocytes were cultured in 96-well tissue culture plates at a density of 2,500 cells per well for 2 days. The wells were then washed with PBS, and Sebomed Complete Medium was added. After 2 days, the medium was harvested, and fresh medium with α-MSH 250 – 15.625 μM and testosterone 10 – 0.625 μM was given to the cells. The supernatants were harvested 48 hr later, the wells were washed twice with PBS, and 100 μL of a 10 μg/mL nile red solution in PBS was added to each well. The plates were then incubated at 37°C for 30 min, and the released fluorescence was read on a HTS Multi-Label Reader(Perkin Elmer LAS, Wellesley, MA, USA). The results are presented as percentages of the absolute fluorescence units in comparison with the controls, using 485 nm excitation and 565 nm emission filters for

neutral lipids and 540 nm excitation and 620 nm emission filters for polar lipids. Experiments were performed in triplicate, with 10 wells evaluated for each data point in each experiment.

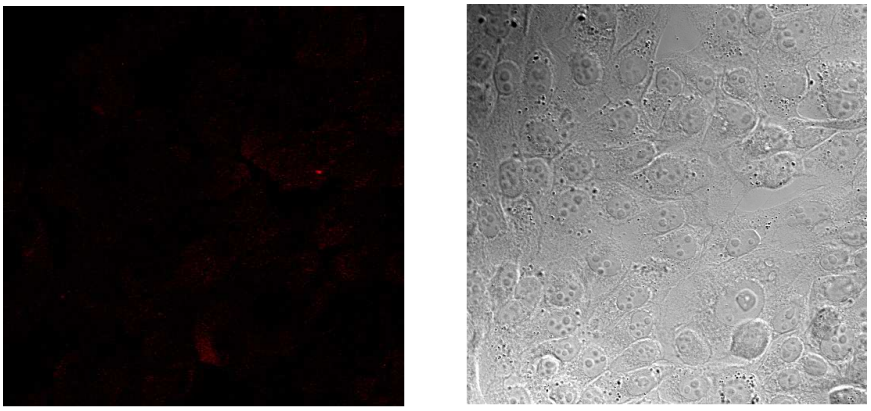
3. Statistical analysis

Statistical analysis using Student's t-test, multiple linear regression analysis and ANOVA were completed using Statistical Product and Service Solutions software (SPSS Inc, Chicago, Ill) for Windows (version 12). A p value of less than 0.05 was considered significant.

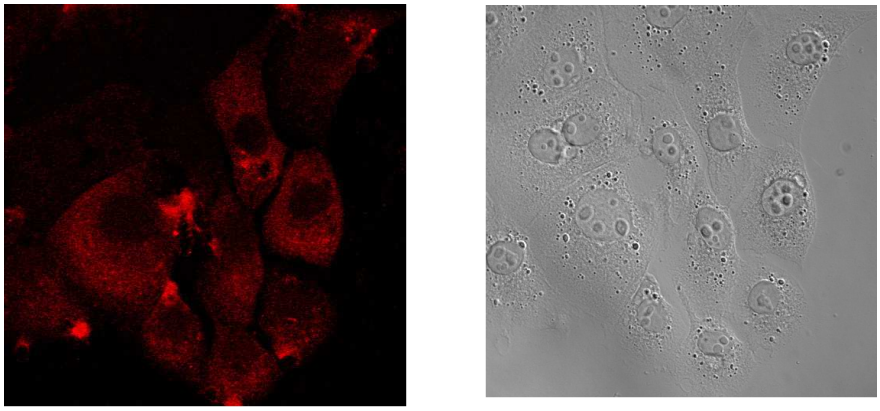
III. RESULTS

1. MC5R expression in differentiated immortalized human sebocyte (SZ95)

SZ95 sebocytes were stimulated to differentiate by testoserone. Expression of MC5R, visualized as punctate fluorescence surrounding the cell membrane, was observed in differentiated SZ95 sebocytes only (Fig 2).



A. Control (Confocal micrographs, X400)

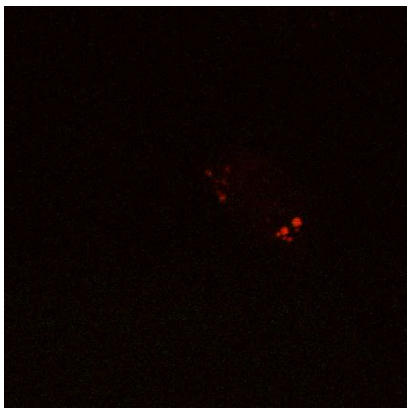


B. Testosterone treated for 2 days (Confocal micrographs, X400)

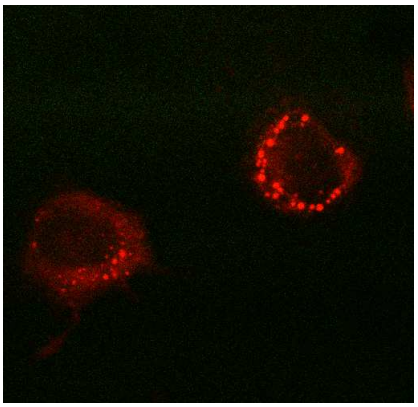
Figure 2. Visualization of MC5R immunoreactivity on the surface of differentiated SZ95 sebocytes(B) and control(A)

2. Sebotropic effect of α-MSH in differentiated immortalized human sebocytes(SZ95)

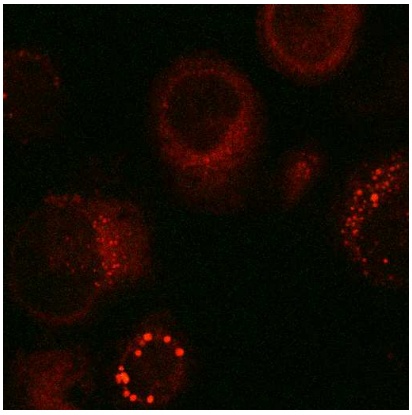
Nile red, a fluorescent probe for lipids, was used to demonstrate the lipids in SZ95 sebocytes. After a 2 day treatment with testosterone, α-MSH, or a mixture of both, results showed a prominent lipid increase in SZ95 sebocytes in the testosterone and α-MSH treatment groups compared to the control and a more prominent lipid increase in the mixed group than in the testosterone or α-MSH group alone (Fig 3).



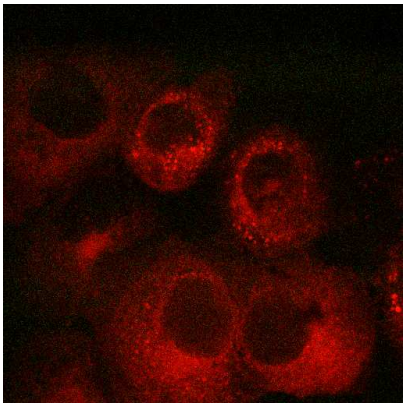
A. Control



B. Testosterone



C. α-MSH



D. Mixture

Figure 3. . Nile red stain after control(A), testosterone(B), α-MSH(C), and mixed(D) treatment. After a 2-day treatment of testosterone (B) or α-MSH(C) lipid depositions were more prominent than control (A) and stronger intensities were also seen after the mixed 2-day treatment (D).

α-MSH and testosterone up-regulate the lipids in a dose dependent manner ($p < 0.05$) (Fig 4).

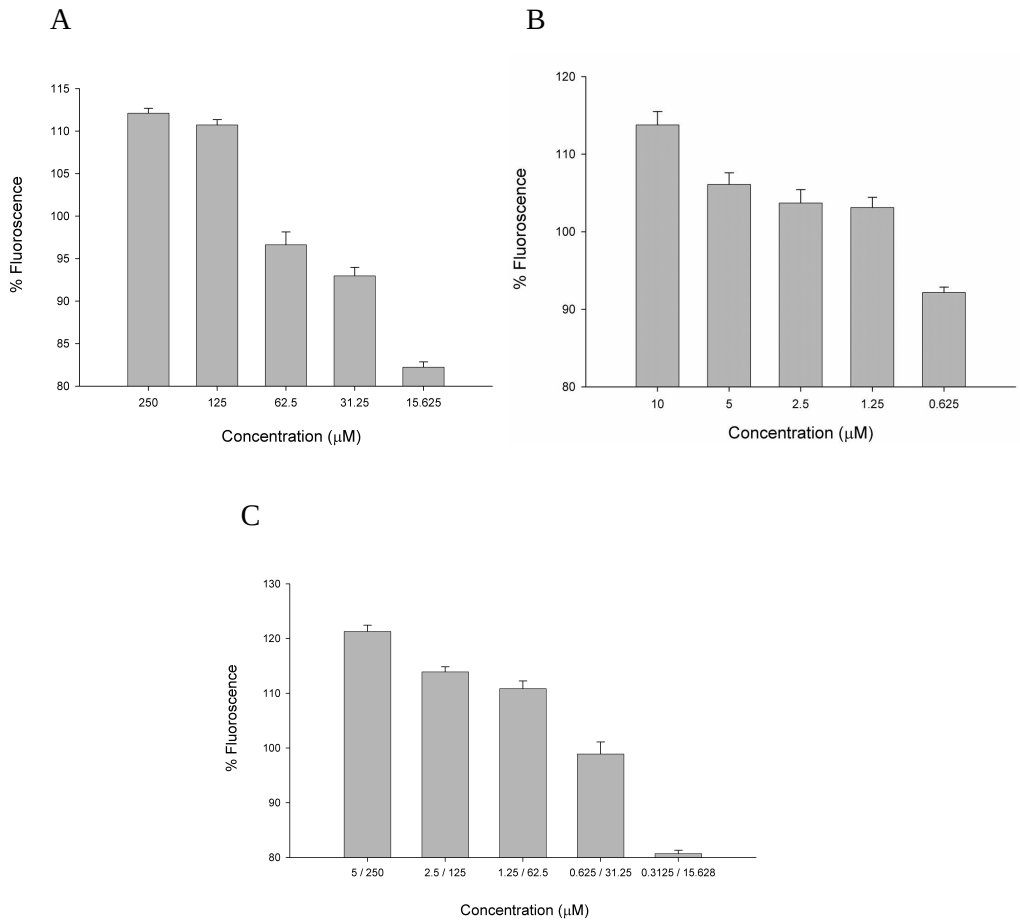


Figure 4. α-MSH(A), testosterone(B), and mixed(C) influence on sebaceous lipogenesis in a dose dependent manner ($P < 0.05$)

3. $[Ca^{2+}]_i$ after α-MSH stimulation

α-MSH produced an immediate increase in $[Ca^{2+}]_i$ in testosterone pre-treated SZ95 sebocytes (*n* = 4 ; Fig 5)

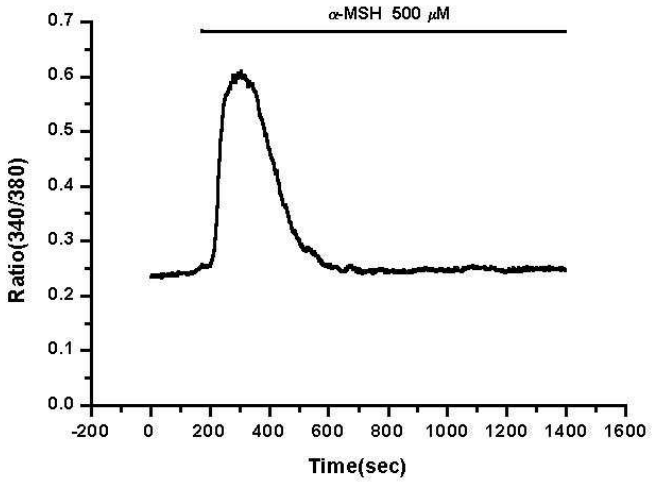


Figure 5. $[Ca^{2+}]_i$ after α-MSH stimulation. Immediate increase in $[Ca^{2+}]_i$ in response to α-MSH

4. $[Ca^{2+}]_i$ after α-MSH stimulation without extracellular Ca^{2+}

It was then examined whether the increases in $[Ca^{2+}]_i$ in response to the α-MSH were dependent upon an influx of extracellular Ca^{2+}. In the absence of extracellular Ca^{2+}, the immediate increase in $[Ca^{2+}]_i$ in response to α-MSH was observed which then returned to its initial level, suggesting that intracellular Ca^{2+} stores were the main source for α-MSH -induced $[Ca^{2+}]_i$ increase. (*n* = 4 ; Fig. 6).

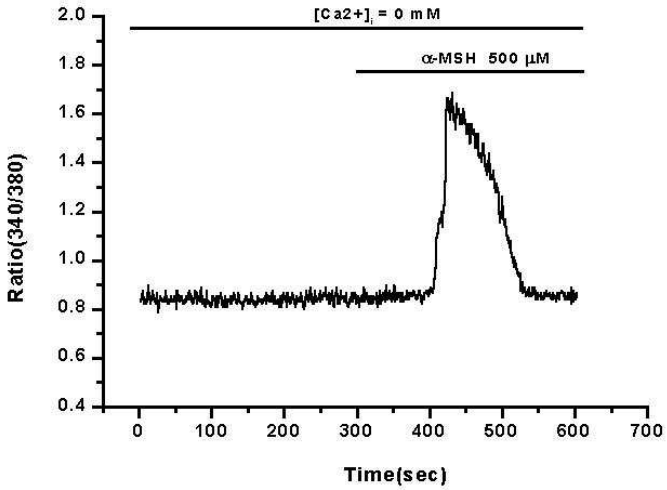
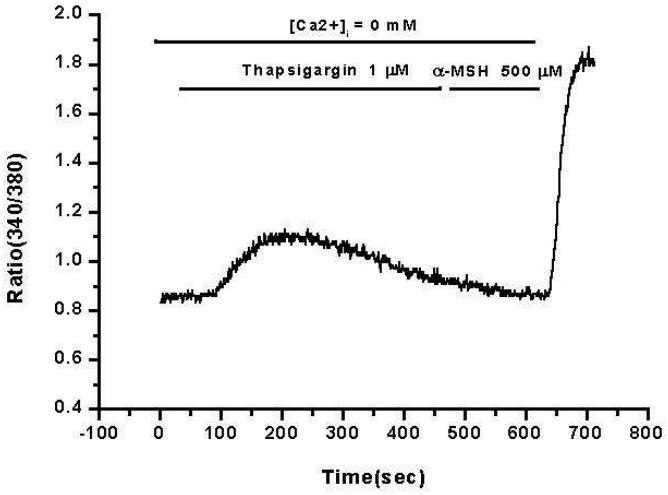


Figure 6. $[Ca^{2+}]_i$ after α-MSH stimulation without extracellular calcium. Transient increase in $[Ca^{2+}]_i$ in response to α-MSH in absence of extracellular Ca^{2+}

5. $[Ca^{2+}]_i$ after thapsigargin treatment, α-MSH stimulation and thapsigargin control

Depletion of the intracellular Ca^{2+} stores with thapsigargin, a specific inhibitor of endoplasmic reticulum (ER), prevented α-MSH induced $[Ca^{2+}]_i$ increase, indicating that the intracellular Ca^{2+} stores responsible for the $[Ca^{2+}]_i$ increase were thapsigargin-sensitive (n = 5 ; Fig 7).

A.



B

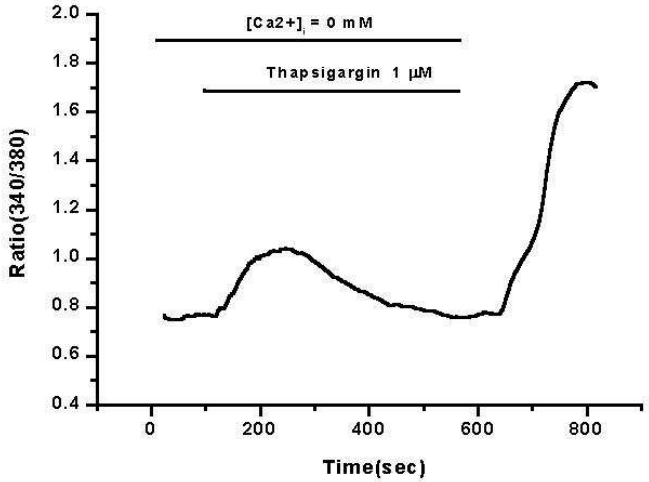


Figure 7. $[Ca^{2+}]_i$ after thapsigargin treatment, α-MSH stimulation(A) and thapsigargin control(B). $[Ca^{2+}]_i$ does not increase in response to α-MSH stimulation and thapsigargin. $[Ca^{2+}]_i$ increase after regular solution infusion.

6. $[Ca^{2+}]_i$ after caffeine treatment and α-MSH stimulation

To examine whether the thapsigargin-sensitive intracellular Ca^{2+} store was releasable by IP3 receptors, cells were exposed to 10 mM caffeine (IP3 receptor antagonists). Caffeine completely prevented the increase of $[Ca^{2+}]_i$ evoked by α-MSH (*n* = 3 ; Fig 8).

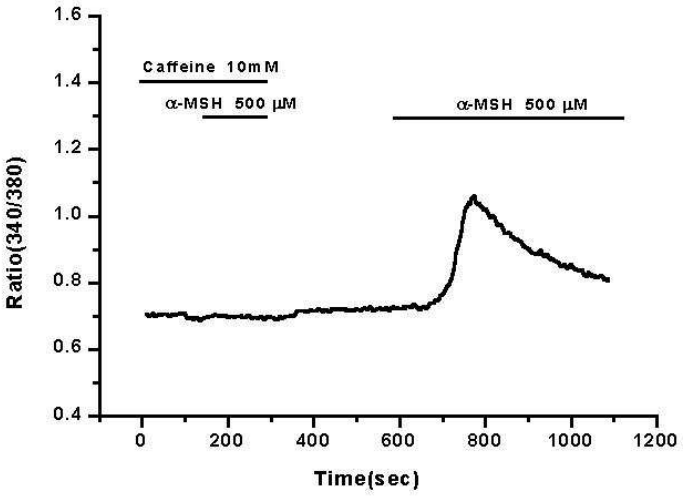


Figure 8. $[Ca^{2+}]_i$ after caffeine treatment and α-MSH stimulation. Caffeine prevented the increase of $[Ca^{2+}]_i$ evoked by α-MSH

7. $[Ca^{2+}]_i$ after 2-APB treatment and α-MSH stimulation

To examine whether the thapsigargin-sensitive intracellular Ca^{2+} store was releasable by IP3 receptors, cells were exposed 75 μM 2-APB (IP3 receptor antagonist). 2-APB completely prevented the $[Ca^{2+}]_i$ increase evoked by α-MSH (*n* = 5 ; Fig 9).

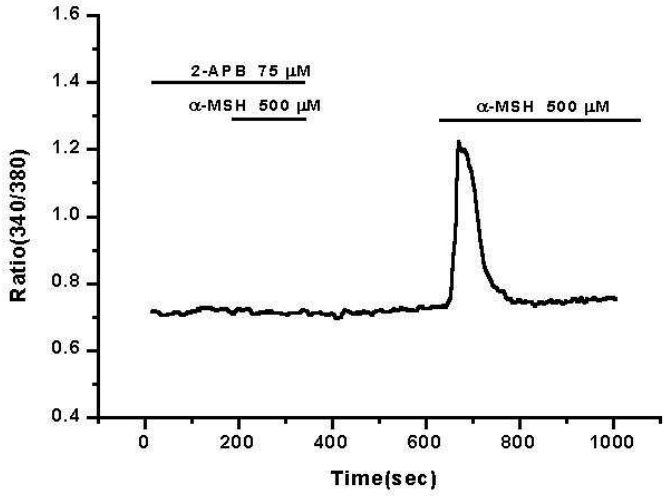


Figure 9. $[Ca^{2+}]_i$ after 2-APB treatment and α-MSH stimulation. 2-APB prevented the $[Ca^{2+}]_i$ increase evoked by α-MSH.

8. $[Ca^{2+}]_i$ after high concentration ryanodine treatment and α-MSH stimulation

Ryanodine failed to inhibit the α-MSH -induced $[Ca^{2+}]_i$ increase. These results suggest that thapsigargin-releasable, IP3-sensitive Ca^{2+} stores are responsible for α-MSH -induced Ca^{2+} mobilization (*n* = 4 ; Fig 10).

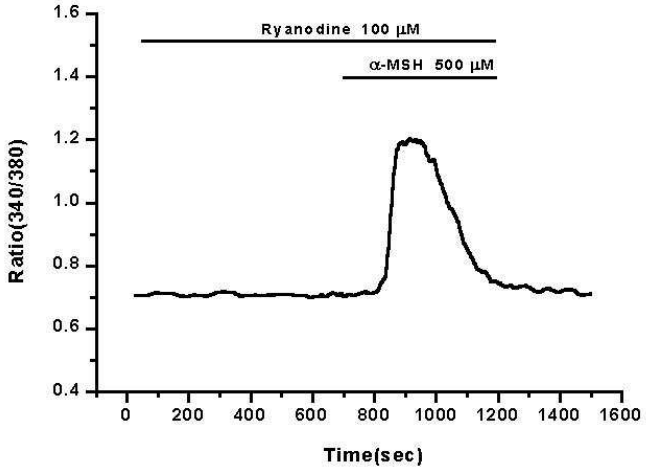
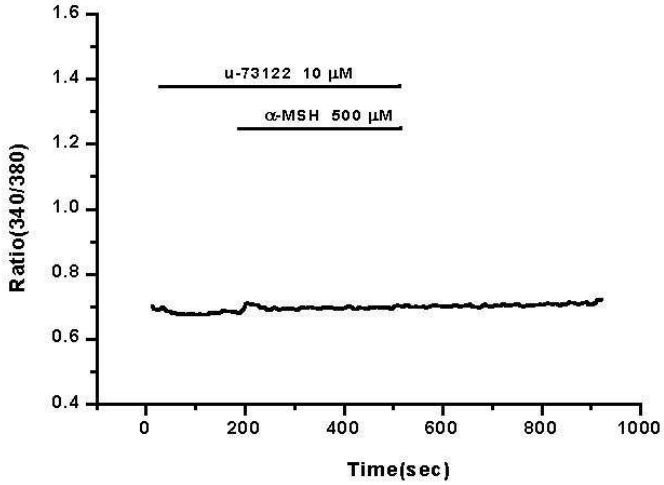


Figure 10. $[Ca^{2+}]_i$ after high concentration ryanodine treatment and α-MSH stimulation. Ryanodine failed to inhibit the α-MSH-induced $[Ca^{2+}]_i$ increase.

9. $[Ca^{2+}]_i$ after PLC inhibitor (u-73122), PLC control (u-73343) treatment and α-MSH stimulation

To further clarify the involvement of PLC in the generation of the $[Ca^{2+}]_i$ increase, we used the PLC inhibitor u-73122 and its inactive analogue u-73343 as control. u-73122, but not u-73343(10 μM), prevented the α-MSH-evoked $[Ca^{2+}]_i$ increase (*n* = 4 ; Fig 11A, *n* = 4 ; Fig 11B).

A



B

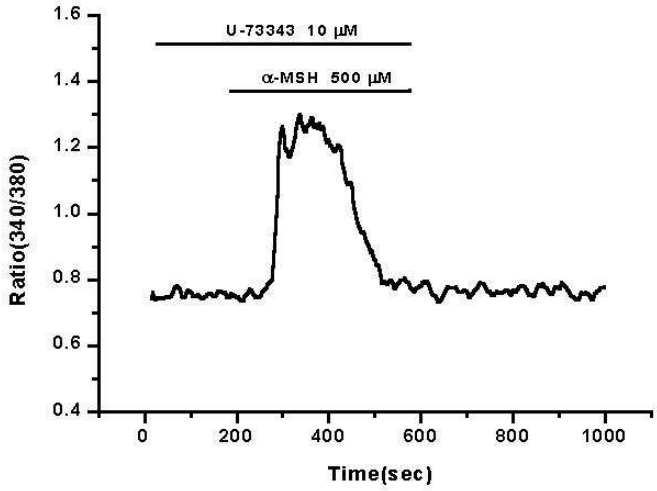


Figure 11. $[Ca^{2+}]_i$ after PLC inhibitor(u-73122)(A), PLC control(u-73343)(B) treatment and α-MSH stimulation. PLC inhibitor(u-73122) blocked $[Ca^{2+}]_i$ increase.

IV. DISCUSSION

Author expected that SZ95 sebocytes could express MC5R and clearly showed the MC5R expression in differentiated SZ95 sebocytes, because human sebocytes and primary cultured sebocytes express MC1R and MC5R according to their cell differentiation state; undifferentiated SZ95 sebocytes express MC1R[5]. In human skin, MC5R was detected only in differentiating, lipid-laden sebaceous cells but not in basal, undifferentiated sebaceous cells. Similarly, in cultured human sebocytes, MC5R was only detectable at the onset of differentiation and in fully differentiated cells, displaying prominent lipid granules. MC5R is a regulator of sebum secretion control and a marker of human sebocyte differentiation[6].

The HEK 293 cells transfected with mouse MC5R showed a-MSH-stimulated $[Ca^{2+}]_i$ increase and suggested the possible intracellular calcium increase channel.[10, 11] Authors′ experiments showed α-MSH-stimulated $[Ca^{2+}]_i$ increase, and this suggests the possibility that Ca^{2+} could also be a possible signaling molecule in SZ95 sebocytes.

Whereas the production of cAMP via Gs-protein stimulation of adenylate cyclase is a well established pathway for the MC5R[3], the mechanisms involved with the elevation of $[Ca^{2+}]_i$ are less understood.

In most cells, Ca^{2+} has its major signaling function when its concentration is elevated in the cytosolic compartment.[14-17] From there it can also diffuse into the nucleus[18] or be sequestered by mitochondria.[19] The Ca^{2+} concentration inside cells is regulated by the simultaneous interplay of multiple counteracting processes, which can be divided into Ca^{2+} ‘on’ and ‘off’ mechanisms, depending on whether they serve to increase or decrease cytosolic Ca^{2+}.[17, 20]

In the present study, author demonstrate that an α-MSH-induced $[Ca^{2+}]_i$ increase was sustained in the absence of extracellular Ca^{2+}, indicating that intracellular Ca^{2+} stores were primarily responsible for the generation of

$[Ca^{2+}]_i$ increase. The two main intracellular organelles containing large amounts of Ca^{2+} are the endoplasmic reticulum and mitochondria.[20] Our data showed that depletion of intracellular Ca^{2+} stores with thapsigargin completely prevented the generation of α-MSH-stimulated $[Ca^{2+}]_i$ increase, suggesting that the thapsigargin-sensitive endoplasmic reticulum Ca^{2+} store was the source of $[Ca^{2+}]_i$ increase.

In the present study, a high concentration of ryanodine (100 μM) failed to prevent the α-MSH-stimulated $[Ca^{2+}]_i$ increase. Instead, 2-APB and caffeine, both IP3-sensitive Ca^{2+} channel inhibitors, blocked the α-MSH-stimulated $[Ca^{2+}]_i$ increase. 2-APB and caffeine have several cellular targets. 2-APB blocks IP3-sensitive Ca^{2+} channels, sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity, and capacitative Ca^{2+} entry channels. Caffeine can stimulate ryanodine receptors, inhibit both cAMP degradation and PLC activation, and prevent IP3-sensitive Ca^{2+} channel opening.[21] However, the only feature that caffeine and 2-APB share is their ability to antagonize IP3-mediated Ca^{2+} release. Therefore, although neither 2-APB nor caffeine are solely selective for IP3-sensitive Ca^{2+} channels, when used judiciously these pharmacological agents can reveal the specific involvement of IP3 signaling. The results obtained using 2-APB and caffeine support the hypothesis that α-MSH-stimulated $[Ca^{2+}]_i$ increase through the activation of IP3-sensitive Ca^{2+} channels. The above results differ from the HEK 293 cells transfected with mouse MC5R experiments, which had suggested that the elevations in $[Ca^{2+}]_i$ were mediated via ryanodine receptors.[10]

PLC is known to be recruited to the plasma membrane following activation of tyrosine kinase receptors and is activated by a mechanism that relies on tyrosine phosphorylation.[22] The phosphorylated PLC catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce diacylglycerol and IP3, leading to the activation of protein kinase C and an increase in $[Ca^{2+}]_i$, respectively.[23] U73122 almost completely prevented α-MSH-stimulated $[Ca^{2+}]_i$ increase but U73343 did not. These results suggest that the production

of IP3 following the activation of PLC is essential for the generation of an α-MSH-stimulated $[Ca^{2+}]_i$ increase in SZ95 sebocytes.

The sebaceous gland has long been recognized as a target for α-MSH.[24, 25] Removal of the posterior pituitary reduces the lipid secretion rates in the rat. The lipotrophic substance responsible for this effect was identified as α-MSH.[26, 27] Further studies have revealed that daily treatment with α-MSH resulted in a dose-dependent increase in sebum secretion of intact rats.[28, 29] Our nile red staining results correspond with previous reports and also showed sebaceous lipogenesis with testosterone and α-MSH in a dose dependent manner.

In this study, I demonstrated testosterone-induced MC5R expression in SZ95 sebocytes and showed that α-MSH stimulation in differentiated SZ95 sebocytes induced PLC activation and $[Ca^{2+}]_i$ elevation. To our knowledge, this is the first report to show the involvement of α-MSH-stimulation-produced PLC-activated Ca^{2+} signaling in differentiated SZ95 sebocytes.

Although other authors have suggested that lipogenesis produced by α-MSH stimulation could be caused by Ca^{2+} signaling, the casual relationship between $[Ca^{2+}]_i$ elevation and lipogenesis needs more investigation. SZ95 sebocytes have morphologic, functional, and phenotypic characters of human sebocytes, but we expect that another Ca^{2+} signaling investigation using primary cultured human sebocytes would give us more information.

I also suggest further investigations into Ca^{2+} signaling of other hormones and neurotransmitters stimulating MC5R, the relationship between cAMP and the Ca^{2+} signaling pathway, and whether Ca^{2+} signaling is a real lipogenesis pathway or not.

V. CONCLUSION

Differentiated sebocytes induced by testosterone express MC5R. α-MSH stimulation induces an immediate increase in $[Ca^{2+}]_i$ in SZ95 sebocytes with or without extracellular Ca^{2+}. Thapsigargin, a specific inhibitor of endoplasmic reticulum(ER), prevented α-MSH induced $[Ca^{2+}]_i$ increase. 2-APB and caffeine, both IP3-sensitive Ca^{2+} channel inhibitors, blocked the α-MSH-stimulated $[Ca^{2+}]_i$ increase, but a high concentration of ryanodine failed to prevent the α-MSH-stimulated $[Ca^{2+}]_i$ increase. α-MSH-stimulated $[Ca^{2+}]_i$ increase was almost completely prevented by PLC inhibitor, U73122, but not by control U73343. We therefore conclude that α-MSH-stimulated $[Ca^{2+}]_i$ increase is caused by activating PLC, through thapsigargin-releasable, IP3-sensitive Ca^{2+} channels and might be a signal of lipogenesis.

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Abstract (In Korean)

α-MSH 투여 시 immortalized sebocyt 의
intracellular calcium 농도의 변화

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여드름의 발병원인 중 가장 중요한 것이 피지선에서 분비되는 피지의 과분비로 알려져 있다. 현재까지 많은 연구에 의하여 피지 분비를 조절하는 인자는 androgen/estrogen, IGF/GF/insulin, PPAR, retinoid, melanocortin 등이 알려져 있다.
여러 연구에서 stress와 관련된 피지분비증가와 관련된 기전으로 POMC에 의해서 생성된 ACTH 1-17, ACTH1-10, α-MSH 들이 분화된 sebocyte에 존재하는 MC5R에 결합하여 피지분비증가를 유발하는 것이 밝혀졌다. 또한 최근에 mouse MC5R gene을 주입하여 MC5R을 표현하는 HEK293 cell에서 melanocortin으로 MC5R를 activation 시켰을 때 intracellular calcium과 cAMP가 증가하는 것을 관찰하여 calcium ion이 피지분비증가와 피지세포분화의 신호전달 물질임을 제시하였다. 또 다른 최근 연구에서는 분화된 sebocyte에서만 MC5R가 발현되어 MC5R이 sebocyte differentiation marker라 하였으며 sebocyte 분화에 cAMP가 필요하다는 연구도 있었다
실험을 통하여 differentiated SZ95 sebocyte에서 MC5R이 표현됨을 관찰하였고 α-MSH 투여에 의한 $[Ca^{2+}]_i$ 증가는 PLC activation과

연관되어 endoplasmic reticulum 등에 저장된 Ca^{2+}이 IP3-sensitive Ca^{2+} channel을 통해 분비되는 기전에 의해 이루어짐을 보여주었다. 또한 α-MSH과 testosterone을 혼합하여 SZ95 sebocyte에 투여하였을 때 각각의 투여 농도에 비례하여 피지생성이 증가함을 보여주었다.

핵심되는 말 : calcium, α -MSH, sebocyte, 피지