Calcium Stimulates Mitochondrial Biogenesis in Human Granulosa Cells

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ABSTRACT: Ovarian granulosa cells are known to play a key role in regulating ovarian physiology. Age increases apoptosis in follicular granulosa cells and subsequently decreases ovarian fecundity. The aging ovary also contains fewer follicles that possess fewer granulosa cells. The viability of follicular granulosa cells may be essential for development of the oocyte. Calcium ion plays an important role in a variety of biological processes, including gene expression, cell cycle regulation, and cell death. To study the ability of mitochondrial biogenesis in human granulosa cells, we determined the mitochondrial marker proteins, including the nuclear-encoded NADH-ubiquinone oxidoreductase alpha subunit 9 (NDUFA9) and mitochondrial-encoded COX I, after treatment of the cells with the calcium ionophore A23187. We showed that the expression of these mitochondrial marker proteins in human granulosa cells increased with changes in cytosolic Ca^{2+} using the ionophore A23187. Treatment of granulosa cells with 0.5 μM of A23187 for 120 h increased the levels of NDUFA 9 and COX I subunit by up to 2.6- and 2.4-fold, respectively. Raising Ca^{2+} by exposing granulosa cells to 1 μM of A23187 for 48 h significantly increased mitochondrial transcription factor (mtTFA) gene expression by up to 2.9-fold. Our results indicate that the adaptive responses of granulosa cells to increased Ca^{2+} may include upregulation of mitochondrial proteins and that mtTFA may be involved in such a mitochondrial biogenesis pathway.

KEYWORDS: calcium; granulosa cell; mitochondria

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Ann. N.Y. Acad. Sci. 1042: 157–162 (2005).
doi: 10.1196/annals.1338.017

INTRODUCTION

Ovarian granulosa cells play a major role in regulating ovarian physiology, including ovulation and luteal regression.[1] Granulosa cells secrete a wide variety of growth factors that may attenuate the action of gonadotrophin in the ovary in paracrine-autocrine processes.[2,3] Most of these factors do not directly affect the oocyte but exert their action via granulosa cells. The presence of granulosa cells appears to be beneficial for oocyte maturation and early development.[4] Nevertheless, granulosa cells might also have a negative effect on the oocyte. It has been demonstrated that the increased apoptotic potential in oocytes of aged mice is caused by the presence of granulosa cells.[5] The decline in reproductive ability with age in women is associated with a loss of follicles and a decrease in oocyte quality. Aging-associated apoptosis increases in follicular granulosa cells and consequently decreases ovarian fecundity.[6,7]

In eukaryotic cells, mitochondria are specialized organelles that catalyze the formation of ATP. Two distinct genomes exist in all eukaryotic cells. One is located in the nucleus and is transmitted in a Mendelian fashion, whereas the other is located in mitochondria and is transmitted by maternal inheritance. The mitochondria in an oocyte must have produced and stored all the energy required for the resumption of meiosis II, fertilization, and development of the embryo.[8,9] Deficiency in mitochondrial ATP production may be associated with impairment of oocyte fertilization.[10,11] Normal function of mitochondria in follicular granulosa cells may be needed for growth factor production and subsequent paracrine effects in oocyte development. Calcium ion plays an important role in a variety of biological processes, including gene expression, cell cycle regulation, and cell death. In this study, the ability of mitochondrial biogenesis in human granulosa cells was determined by incubation of the cells with calcium ion.

MATERIALS AND METHODS

Collection of Human Granulosa Cells

We collected human granulosa cells from patients undergoing *in vitro* fertilization by gonadotrophin-stimulated cycles. This study was approved by the Institutional Review Board of Taipei Medical University Hospital. Follicular fluids aspirated from each patient were pooled and were centrifuged for 10 min at $800 \times g$ at room temperature.

Cell Cultures and Drug Treatment

A23187 was purchased from Sigma (St. Louis, MO). It was prepared as a stock solution in Me_2SO at concentrations of 0.25, 0.5, and 1 μM. Granulosa cells were cultured with HTF medium containing 2% human plasma in an incubator at 37°C. The dose-dependent effects of A23187, from 0.25 to 1 μM, on mitochondrial respiratory chain subunits were evaluated. Total RNA extracted from harvested cells was used as templates, and cDNA was prepared using the RNA extraction kit and reverse-transcription polymerase chain reaction kit from Ambion (Austin, TX).

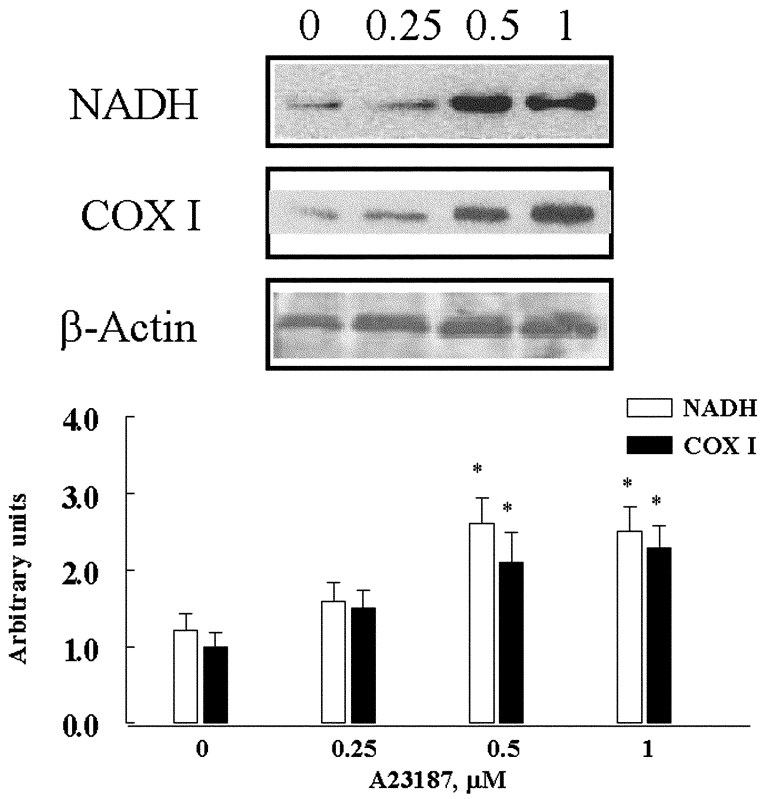

FIGURE 1. Western blots of nuclear- and mtDNA-encoded proteins in human granulosa cells treated for 96 h with A23187 at concentrations ranging from 0.25 to 1 μM.

RESULTS

To study the effects of calcium ion on mitochondrial biogenesis, the mitochondrial marker proteins were determined. Western blot analysis was used to determine the effect of A23187 treatment on the nuclear-encoded NADH-ubiquinone oxidoreductase alpha subunit 9 (NDUFA9) and mitochondrial-encoded COX I levels. A23187 caused a dose-dependent increase in NDUFA9 and COX I levels of up to 2.5- and 2.3-fold ($P < 0.05$; FIG. 1). The effect of A23187 on both the nuclear- and mitochondrial-encoded subunits was also time dependent (FIG. 2). A23187 exposure for 120 h increased NDUFA9 and COX I levels by 2.6- and 2.4-fold, respectively ($P < 0.05$). The possible role of mitochondrial transcription factor A (mtTFA) in mitochondrial biogenesis also was examined. A23187 exposure for 24 and 48 h at 0.5 and 1 μM resulted in an increase in mtTFA mRNA levels by 2.5- (0.5 μM, 24 h), 2.9- (0.5 μM, 48 h), 3.1- (1 μM, 24 h), and 2.9-fold increases (1 μM, 48 h). Taken together, these data show calcium-dependent increases in both nuclear-encoded NDUFA9

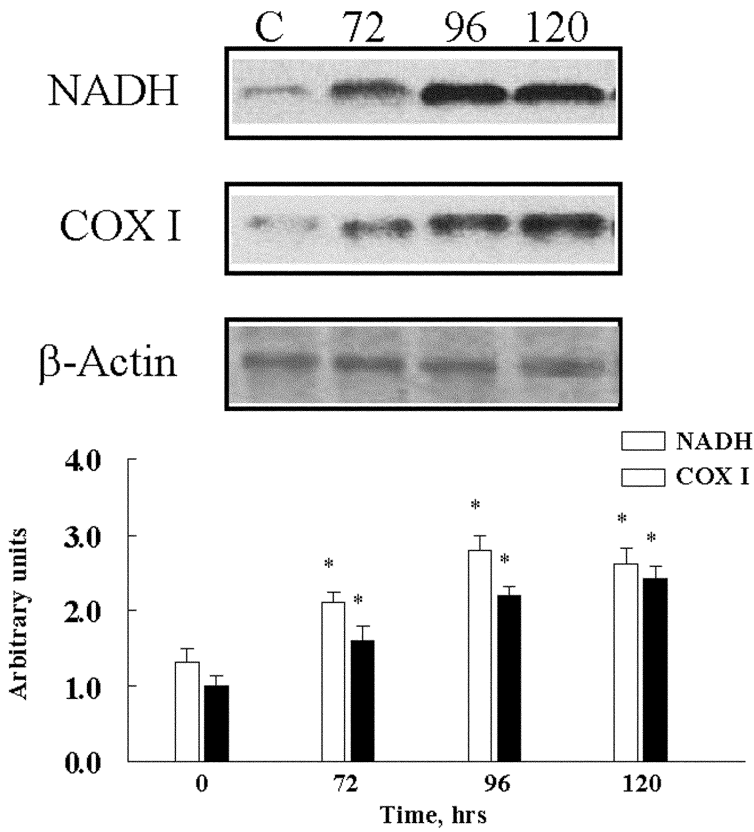

FIGURE 2. Western blots of nuclear- and mtDNA-encoded proteins in human granulosa cells treated with 0.5 μM of A23187 for 72 to 120 h.

and mitochondrial-encoded COX I expression levels and suggest that mtTFA may be involved in this process.

DISCUSSION

Mitochondrial biogenesis requires the symphonious expression of mtDNA and nuclear genes that both encode mitochondrial proteins and their regulatory factors. One of these factors is mtTFA, which activates mtDNA transcription and replication.[12] Cellular responses to environmental changes including energy demands should be reflected in changes in the physiological state of mitochondria. Our data indicate increased expression levels of mtTFA mRNA in human granulosa cells after Ca^{2+} stimulation. This event was accompanied by changes in mitochondrial biogenesis, reflected by an increase in NDUFA9 and COX I levels. These findings are sim-

ilar to an increase in mitochondrial biogenesis induced by Ca^{2+} in muscle,[13] and by mitogens in human lymphocytes[14] and murine splenocytes.[15]

Ca^{2+} acts as a second messenger in a variety of biological processes.[16,17] In this study, the effects of Ca^{2+} on both nuclear- and mitochondrial-encoded subunits were dose and time dependent. Changes in expression levels of mtTFA were also dependent on Ca^{2+}. Previous studies have provided evidence that cytosolic Ca^{2+} concentration is involved in regulating mitochondrial biogenesis in skeletal muscle.[13,18] The adaption of mitochondria in this process involves induction of mtTFA.[13] This experimental evidence indicates that Ca^{2+} upregulates mtTFA expression, leading to mitochondrial biogenesis. Results from this study are in accordance with the contention that Ca^{2+} is one of the signals that mediate mitochondrial biogenesis in differentiated granulosa cells.

ACKNOWLEDGMENTS

This work was supported by research grants NSC 91-2320-B-038-026 and NSC 92-2320-B-038-045 from the National Science Council of the Republic of China.

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