行政院國家科學委員會補助專題研究計畫

V 成果報告　□期中進度報告

（計畫名稱）Adenosine Triphosphate 對人類卵巢顆粒細胞凋亡之影響

計畫類別：V 個別型計畫　□ 整合型計畫

計畫編號：NSC 91 － 2314 － B －038 - 004 －

執行期間： 91 年 03 月 01 日至 91 年 12 月 31 日

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成果報告類型(依經費核定清單規定繳交)：□精簡報告 V 完整報告

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中　華　民　國　92　年　3　月　27　日

中文摘要：

由以往的研究文獻中，我們得知腺嘌呤核甘三磷酸（ATP）會在生物體內的不同系統中，引起細胞凋亡反應，然而 ATP 在人類卵巢顆粒細胞（$hGLC_s$）的影響，所知道的並不多。這實驗設計主要是探討 ATP 對於活化 caspase 系統的細胞內訊息傳遞的路徑和引發細胞凋亡反應所扮演的角色，人類卵巢顆粒細胞是從進行試管嬰兒體外受精的病人之卵泡抽取液中取得，藉由 DMEM 培養基及胎牛血清在 in vitro 進行三天的細胞培養，之後再進行我們的研究。細胞開始處理之前，先在沒有血清之培養液中培養 4 小時，為了解 ATP 不同濃度之影響，顆粒細胞以 10 μM、100 μM、1mM or 10mM 處理 24 小時；在 time-course 的實驗中，$hGLC_s$ 以 10mM ATP 處理 6、12 或 24 小時。之後進行 Western blot 分析，我們使用可測得 caspase-3、-9 or PARP 的抗體。引起細胞凋亡反應的定量方法，我們是以免疫酵素分析法（enzyme linked immunosorbent assay；ELISA）去測 DNA fragmentation 的量。先前，我們曾發表 ATP 會引發一連串的 $hGLC_s$ 內訊息傳遞系統，並且會調控人類絨毛膜刺性腺激素（human chorionic gonadotropin；hCG）對卵巢的作用，為檢測 hCG 因 ATP 引起凋亡的反應中 hCG 所扮演的角色，我們在 $hGLC_s$ 中加入 10 IUhCG 及 10mM ATP 共同培養 12 小時。

從 dose- and time-dependent 的實驗設計中，確實證明 ATP 會引起 DNA fragmentation，依據西方墨點法（Western blot）的分析，可探偵到 PARP 被活化或者 caspase-3 的 pro- 及被活化的模式；印證 ATP 在 $hGLC_s$ 所引起的細胞凋亡反應中，對於 caspase 系統活化的細胞內訊息傳遞的路徑，引發蛋白？將 pro-caspase-3 轉換為活化的 caspase-3，之後 DNA 的修補？ PARP 產生裂解；而根據我們實驗的觀察，caspase-9 在 ATP 所引起的細胞凋亡反應中並沒有被活化。令人感興趣的是，我們從實驗中發現，在 ATP 所引起的細胞凋亡反應過程中，hCG 可以將其反應減弱。

據我們目前所知，在人類的卵巢中，藉由 ATP 引發 caspase 系統的細胞內訊息傳遞路徑，以往的文獻並沒有相關的探討。本篇研究所得到的結果，支持我們的想法，憑藉神經傳導物質對人類卵巢所產生細胞凋亡反應過程中，確實活化 caspase 系統之路徑，同時也更進一步的了解其作用機轉。

關鍵詞: 腺嘌呤核甘三磷酸（ATP）、卵巢顆粒細胞、細胞凋亡

Abstract

Adenosine triphosphate (ATP) has been shown to induce programmed cell death in various systems. However, little is known about the effect of ATP on hGLCs. The present study was designed to examine the effect of ATP on the activation of the caspase signaling pathway and its role in inducing programmed cell death. Human GLCs were collected from patients undergoing in vitro fertilization programs, and then were cultured in FBS-supplemented DMEM for 3 days prior to our studies. Cells were starved in serum-free medium for 4 h prior to treatment. To examine the dose-response relationship, hGLCs were treated with increasing concentrations of ATP (10 μM, 100 μM, 1 mM or 10 mM) for 24 hours. For time-course experiments, hGLCs were treated with 10 mM ATP for 6, 12, or 24 hours. Western blot analysis was performed using antibodies against the pro- and active forms of caspase-3, -9, or PARP. To quantify the induction of apoptosis, DNA fragmentation was measured using the cell death detection enzyme-linked immunosorbent assay. To examine the effect of human chorionic gonadotropin (hCG) in protecting cells from apoptosis, hGLCs were treated with 10 IU hCG in the presence of 10 mM ATP for 12 hours.

It was demonstrated that ATP was capable of inducing DNA fragmentation in a dose- and time-dependent manner. Furthermore, Western blot analysis, which detected the pro- and active forms of caspase-3, or PARP, demonstrated that ATP activated the caspase-signaling pathway, leading to the proteolytic conversion of pro-caspase-3 to active caspase-3, and the subsequent cleavage of the caspase substrate PARP. Based on our observation, caspase-9 was not triggered by ATP. Interestingly, hCG attenuated the effect of ATP in activating the caspase signaling pathway.

To our knowledge, this is the first demonstration of the ATP-induced activation of the caspase signaling pathway in the human ovary. These results support the notion that the caspase-signaling pathway is involved in mediating ATP actions in the human ovary.

Key word: ATP，granulosa-luteal cells，apoptosis

目錄：

Introduction

Adenosine triphosphate (ATP) is released from cells such as platelets and co-released with neurotransmitter granules from autonomic nerves by exocytosis (1). Extracellular ATP binds to a G protein-coupled P2 purinoceptor that activates phospholipase C and phosphatidylinositol hydrolysis, generating diacylglycerol and inositol 1,4,5-triphosphate, which stimulate protein kinase C and cytosolic calcium ([Ca2+]i) mobilization, respectively (1,2). Thereafter, ATP may participate in various types of physiological responses, including secretion, membrane potential, cell proliferation, platelet aggregation, neurotransmission, cardiac function, and muscle contraction (3,4).

Purinergic receptors have been classified as P1 and P2 receptors. Pharmacologically, the P1 receptors have a high affinity for extracellular adenosine and AMP (Adenosine > AMP > ADP > ATP), whereas P2 receptors have a high affinity for ATP and ADP (ATP > ADP > AMP > adenosine). Six subtypes of P2 purinergic receptors, P2X, P2Y, P2D, P2T, P2Z, and P2U, have been identified in pharmacological and molecular cloning studies (1,4).

Autonomic nerves have been shown to innervate the ovary (5). It has been proposed that the co-released ATP from autonomic nerve endings in the ovary may play a role in regulating ovarian function (6). Functionally, a P2U purinoceptor (P2UR) has been detected in human granulosa-luteal cells (hGLCs) using microspectrofluorimetry. Our previous data demonstrated that adenosine triphosphate (ATP) play a crucial role in regulating ovarian functioning by activating the intracellular signaling pathway and modulating human chorionic gonadotropin (hCG) action (7,8).

Apoptosis, or programmed cell death, plays a critical role in the development and homeostasis of all multicellular organisms (9, 10). Apoptosis is characterized by cell shrinkage, membrane blebbing, nuclear pyknosis, chromatin condensation, and genomic fragmentation (11, 12). The aspartate-specific cysteine protease (caspase) cascade appears to be the main pathway by which cellular death is orchestrated (13,14). The involvement of a cysteine protease in apoptosis was firstidentified in the nematode Caenorhabditis elegans (15).

Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme selectively activated by DNA strand damage to participate in DNA repair. PARP catalyzes the successive transfer of ADP-ribose units from its substrate NAD to a variety of proteins, including PARP itself, to produce linear and/or branched homopolymers (16).

ATP has been shown to induce apoptosis in various systems (17), such as the pulmonary artery endothelial cells (18), dendritic cells (19), and mesangial cells (20). Recently, many of the key factors involved in the cell death cascade have been revealed. However, little is known about the signaling pathway of ATP-mediated programmed cell death. In the present study, we examined the effect of ATP on activation of the caspase signaling pathway and PARP in human granulosa-luteal cells (hGLCs). In addition, to determine and quantify the induction of apoptosis by ATP in hGLCs, DNA fragmentation was measured using a cell death detection ELISA kit. As human chorionic gonadotropin (hCG) has been demonstrated to suppress apoptosis in ovarian cells (21-23), the effect of hCG on ATP-induced apoptosis was also examined.

Materials and Methods

Reagents and Materials

ATP was obtained from Sigma Chemical Co. Dulbecco's Modified Eagle Medium (DMEM), penicillin-streptomycin, and fetal bovine serum (FBS) were obtained from GIBCO-BRL. Antibodies against caspase-3, caspase-9, and Poly(ADP-ribose) polymerase (PARP) were purchased from PharMingen. A Cell Death Detection ELISA kit was obtained from Roche Molecular Biochemicals.

Human granulosa-luteal cell cultures

Human GLCs were collected from patients undergoing in vitro fertilization treatment. The use of human GLCs was approved by the Clinical Screening Committee for Research and Other Studies Involving Human Subjects, in the Department of OB/GYN of Taipei Medical University Hospital. Granulosa cells were separated from red blood cells in follicular aspirates by centrifugation through Ficoll Paque, washed, and suspended in DMEM containing 100 U penicillin G/ml, 100 μg streptomycin/ml, and 10% FBS, as previously described (24). The cells were plated at a density of approximately 150,000 cells in 35-mm culture dishes. Cells were then incubated at 37 C under a water-saturated atmosphere of 5% CO_2 in air for 3 days.

Treatment

Human GLCs were incubated in a serum-free medium for 4 h prior to treatment. To examine the dose-response relationship, hGLCs were treated with increasing concentrations of ATP (10 μM, 100 μM, 1mM, or 10mM) for 24 h. For time-course experiments, hGLCs were treated with 10 mM ATP for 6, 12, or 24 h.

To examine the effect of human chorionic gonadotropin (hCG) on protecting cells from apoptosis, hGLCs were treated with 10 IU hCG in the presence of 10 mM ATP for 12 hours.

Western blot analysis

The hGLCs were collected after treatment and lysed with 100 μL of cell lysis buffer (RIPA, 150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 1 % Nonidet P-40, 0.5 % deoxycholate, 0.1% SDS, 1.0 mM PMSF, 10 μg/mL leupeptin and 100 μg/mL aprotinin) at 4 C for 30 min. The cell lysate was centrifuged at 10,000 x g for 5 min and the supernatant was collected for Western blot analysis. The amount of protein was quantified using a Bio-Rad protein assay kit (Bio-Rad Laboratories), following the manufacturer's protocol. Aliquots (30 μ g) were subjected to 10 % SDS-polyacrylamide gel electrophoresis under reducing condition, as previously described (25). The proteins were electrophoretically transferred from the gels onto nitrocellulose membranes (Amersham Pharmacia Biotech), based on the procedures of Towbin et al. (26). These nitrocellulose membranes were probed with a mouse monoclonal antibody or a rabbit polyclonal antibody directed against caspase-3, -9, or PARP, respectively at 4 C for 16 h. After washing, the membranes were incubated with HRP-conjugated secondary antibody, and the signal was visualized using ECL system (Amersham Pharmacia Biotech), followed by autoradiography. The autoradiograms were quantified using a laser densitometer.

Determination of cytoplasmic histone-associated DNA fragments by ELISA kit

To determine and quantify the induction of apoptosis by ATP in hGLCs, DNA fragmentation was measured using a Cell Death Detection ELISA kit (Roche Molecular Biochemicals). Briefly, after treatment with increasing concentrations of ATP (10 μM – 10 mM), one μg of cell lysate was used for the ELISA procedure, following the manufacture's protocol. DNA fragmentation was quantified at 405 nm.

Statistical analysis

The activities of caspase-3, -9, or PARP were expressed as a relative ratio of basal levels. DNA fragments were demonstrated as an O.D. reading. Independent replicates of experiments in this study were performed with cells from three different patients. Data were represented as means ± standard error (SE). Statistical analysis was performed by one-way analysis of variance, followed by Tukey's multiple comparison test. Differences were considered significant at $p < 0.05$.

Results

Effect of ATP on inducing DNA fragmentation in hGLCs

Purine nucleosides and nucleotides have been shown to cause apoptosis in various systems. In the present study, hGLCs were treated with increasing concentrations of ATP (10 μM, 100 μM, 1 mM, or 10 mM) for 24 h. Cell lysates were collected for the detection of DNA fragmentation using an ELISA kit. As shown in Fig. 1A, 10 mM ATP was capable of inducing DNA fragmentation. In contrast, lower levels of ATP did not increase DNA fragmentation, compared with that in the control group.

For time-course experiments, hGLCs were treated with 10 mM ATP for 6, 12, or 24 h. As demonstrated in Fig. 1B, DNA fragmentation was elevated after treatment for 24 hours. No significant change was noted at 6 h and 12 h following ATP treatment.

Effect of ATP on activating caspase in hGLCs

The caspase signaling pathway has been demonstrated to play a central role in cellular apoptosis. In the present study, hGLCs were treated with increasing concentrations of ATP (10 μM, 100 μM, 1 mM, or 10 mM) for 24 h. For time-course experiments, hGLCs were treated with 10 mM ATP for 6, 12, or 24 h. Cell lysates were collected for Western blot analysis. Aliquots (30 μg) were subjected to 10% SDS-polyacrylamide gel electrophoresis under a reducing condition, as previously described (14). A rabbit polyclonal antibody directed against the pro- and active caspase-3 was used, and the signal was visualized using an ECL system (Amersham Pharmacia Biotech), followed by autoradiography. As shown in Fig. 2A, 10mM ATP was able to activate caspase-3 by cleaving pro-caspase-3 (32 kD) to active caspase-3 (17 kD). Fig. 2B indicated that 10 mM ATP activated caspase-3 after treatment for 6 h. By contrast, caspase-9 was not triggered by ATP (data not shown).

Effect of ATP on PARP breakdown in hGLCs

PARP, acting intranuclearly to repair damaged DNA, is one of the substrates of activated caspase. In the present study, hGLCs were treated with increasing concentrations of ATP (10 μM, 100 μM, 1 mM or 10 mM) for 24 h. For time-course experiments, hGLCs were treated with 10 mM ATP for 6, 12, or 24 h. Cell lysates were collected for Western blot analysis. As shown in Fig. 3A, 10 mM ATP was able to cleave intact PARP (116 kD) into an 85 kD fragment. Fig. 3B indicates that 10 mM ATP cleaved PARP after treatment for 6 h, and the effect of ATP was presented in a time-dependent manner.

Effect of hCG on ATP-induced apoptosis

Human CG has been demonstrated to play a role in protecting cells from apoptosis. In the present study, hGLCs were treated with 10 IU hCG in the presence of 10 mM ATP for 12 hours. As shown in Figs. 4A and B, hCG clearly attenuated the effect of ATP on caspase-3 activation and PARP breakdown. Further, we have observed that the protective effect of hCG on ATP-induced apoptosis in hGLCs was only transient; progression of apoptosis was evident following 18 h of treatment with ATP even in the concomitant presence of hCG (data not shown).

Discussion

Purines have been shown to induce apoptosis in various systems (17-20). Recently, many of the key factors involved in the cell death cascade have been revealed. Purine nucleosides and nucleotides are mostly released from cells which are highly activated, stressed, anoxic, and injured (27,28). Adenosine appears to act through P1 purinoceptors, whereas ATP may involve both P2X1 and P2X7 purinoceptors (17). In the present study, we examined the signaling pathway of ATP-mediated cell death in human granulosa-luteal cells.

Two distinct forms of eucaryotic cell death- necrosis and apoptosis- can be distinguished by the morphological and biochemical criteria (29, 30). Necrosis is associated with the increased ion permeability of the plasma membrane and decreased intracellular ATP production. The cell swells and the plasma membrane ruptures within minutes. In contrast, apoptosis is characterized by membrane blebbing, cytoplasm condensation, increased intracellular ATP production, and the activation of specific proteases as well as endogenous endonucleases. The endogenous endonuclease could cleave double stranded DNA at the internucleosomal linker region, generating mono- and oligonucleosomes- DNA fragments. Instead, the DNA of the nucleosomes is tightly complexed to the core histones H2A, H2B, H3, and H4, and therefore is protected from damage by endonuclease (31, 32). The DNA fragments are discrete multiples of an 180 bp subunit which is presented as a “DNA-ladder” on agarose electrophoresis. Subsequent to DNA degeneration, the cytoplasma of the apoptotic cells is rich in mono- and oligonucleosomes, and plasma membrane breakdown will ensue in several hours (33-35).

At least 14 distinct mammalian caspases have been discovered, and their orthologs present in species ranging from the nematode to the dipteran Drosophila melanogaster and the lepidopteran Spodoptera frugiperda (36-40). Caspases involved in apoptosis are generally divided into two categories: the initiator caspases, which include caspase-2, -8, -9, and -10, and the effector caspases, which include caspase-3, -6, and -7 (41). Active caspase-3 consists of 17 and 12 kD subunits which are derived from a 32 kD proenzyme (pro-caspase-3). The caspase-3 antibody recognizes both the 32 kD pro-caspase-3 and the 17 kD subunit of active caspase-3 (42). In the present study, ATP was able to activate caspase-3 in a dose- and time-dependent manner by cleaving pro-caspase-3 (32 kD) to active caspase-3 (17 kD). Ten mM ATP was capable of activating caspase-3, while lower concentrations of ATP was not. Ten mM ATP activated caspase-3 after treatment

for 6 h. We have observed that caspase-9 was not activated by ATP.

There are two main pathways leading to the activation of caspases. The first of these depends upon the participation of mitochondria (receptor-independent). Pro- and anti-apoptotic members of the Bcl-2 family regulate the mitochondrial pathway. Cellular stress induces pro-apoptotic Bcl-2 family members, such as Bax, Bak, and Bok, resulting in the release of cytochrome c from mitochondria, while the anti-apoptotic Bcl-2 proteins, such as Bcl-2, Bcl-XL, and Bcl-w, work to prevent cytochrome c release from the mitochondria, and thereby prevent apoptosis (41, 42). Once in the cytoplasm, cytochrome c catalyzes the oligomerization of apoptotic protease activating factor-1, thereby promoting the activation of procaspase-9, which then activates procaspase-3. The second pathway involves the interaction of a death receptor with its ligand. Receptor-dependent pathways involve the activation of procaspase-8. The active caspase may either activate procaspase-3 or subsequently induces cytochrome c release from mitochondria (45).

PARP is a 116 kD nuclear enzyme that converts NAD to nicotinamide and protein-linked ADP-ribose polymers, the functioning of which is important for DNA repair and genomic maintenance. In response to DNA damage, PARP activity increases, resulting in the poly-(ADP) ribosylation of many nuclear proteins, including PARP itself. In cells, which are destined to undergo apoptosis, the 116 kD PARP protein becomes cleaved by a cysteine protease, caspase-3, producing 85 kD and 25 kD fragments, and results in the loss of normal PARP functions (46, 47).

DNA repair and apoptosis are two essential systems in humans which maintain genomic integrity. There are five major DNA repair pathways: homologous recombinational repair (HRR), non-homologous end joining (NHEM), nucleotide excision repair (NER), base excision repair (BER), and mismatch repair (MMR) (48). Poly(ADP-ribose) polymerase (PARP) is responsible for BER. One of the immediate cellular responses to DNA damage is the activation of PARP, which occurs upon binding to single- or double-strand breaks in the DNA, probably by recruiting DNA repair enzymes to the damaged DNA region (49-51). PARP catalyzes the synthesis of poly(ADP-ribose) from the respiratory coenzyme NAD+ with the release of nicotinamide (NAM). The branched linear ADP polymer is attached to PARP itself, p53, Ca++/Mg++ enconuclease, and to other nearby DNA binding proteins such as histones. Because of its negative charges, the attachment of ADP-ribose polymers to acceptor proteins creates a negatively charged region around the break, thereby opening up the chromatin and allowing the access of repair proteins to the site of the

DNA lesion (52,53).

Since PARP consumes NAD+ and ATP, excessive DNA damage will ultimately lead to cell death through highly evoked PARP activity (54). PARP is cleaved by caspase-3, suggesting that PARP-mediated BER is suppressed once a cell is destined to apoptosis (55). Our findings revealed that ATP induced caspase-3 activation and a PARP breakdown, culminating in the death of human granulose-luteal cells.

Human chorionic gonadotropin (hCG) has been demonstrated to suppress apoptosis in various ovarian cells, such as the preovulatory follicular cells, ovarian cancer cells, and ovarian surface epithelial cells (21-23). Up-regulation of insulin-like growth factor-1 may play a role in mediating the apoptotic effect of hCG in ovarian cells (22). Our previous study revealed that ATP exerts its antigonadotropic action by reducing hCG-induced progesterone production and cyclic AMP accmulation through protein kinase C activation (7,8). In the present study, the effect of hCG on ATP-induced apoptosis was examined. Our results demonstrate that hCG was capable of postponing the effect of ATP in activating the caspase-PARP cascade. However, based on our observation, the progression of apoptosis is inevitable, even in the presence of hCG.

Investigators have reported that ATP may act as a trigger for apoptosis (56) and that ATP at a concentration of 2.0 mM causes cell death in the ovary (57). Our previous study revealed that hCG down-regulated the P2U pruinoceptor in hGLCs (6). It is conceivable that hCG is capable of minimizing the detrimental effect of ATP, at least in part, by the down-regulation of the P2U receptor expression in hGLCs.

In summary, our results revealed that (1) ATP is capable of inducing DNA fragmentation at a 10mM level, (2) ATP is able to activate caspase-3, and (3) PARP, which is responsible for DNA repair, is cleaved into fragments. To our knowledge, this is the first demonstration of the ATP-induced activation of the caspase-signaling pathway in the human ovary. These results support the notion that the caspase-signaling pathway is involved in mediating ATP actions in the human ovary, and can enrich our knowledge regarding the relationship between neuroendocrine and the ovarian function.

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Fig.1A ATP induced apoptosis of human granulose-luteal cells (hGLCs) in a dose-dependent manner. Human GLCs were incubated in a serum-free medium for 4 h prior to treatment, and were treated with increasing concentrations of ATP (10 μM, 100 μM, 1 mM, or 10 mM) for 24 h. To determine and quantify the induction of apoptosis by ATP in the hGLCs, one μg of cell lysate was used for the Cell Death Detection ELISA kit. DNA fragmentation was quantified at 405 nm. Data were represented as means ±standard error (SE). Differences were considered significant at $p < 0.05$.

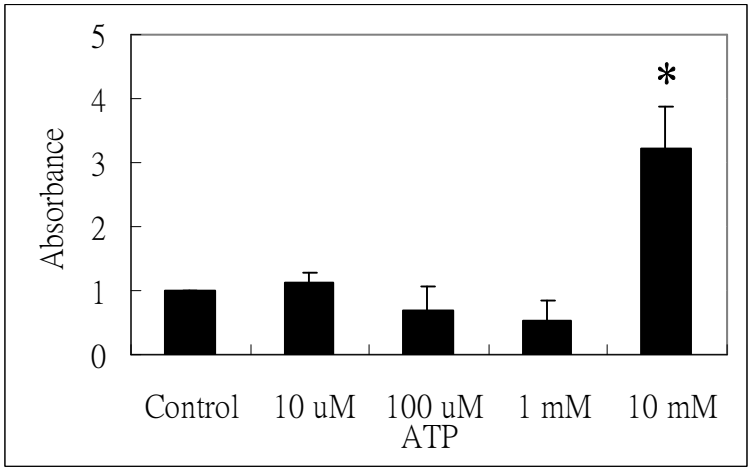


Fig.1B ATP induced apoptosis of human granulose-luteal cells(hGLCs) in a time-dependent manner. Human GLCs were incubated in serum-free medium for 4 hours prior to treatment, and were treated with 10 mM ATP for 6, 12, or 24 hours. To determine and quantify the induction of apoptosis by ATP in hGLCs, one μg of cell lysate was used for the Cell Death Detection ELISA kit. DNA fragmentation was quantified at 405 nm. Data were represented as means ± standard error (SE). Differences were considered significant at $p < 0.05$.

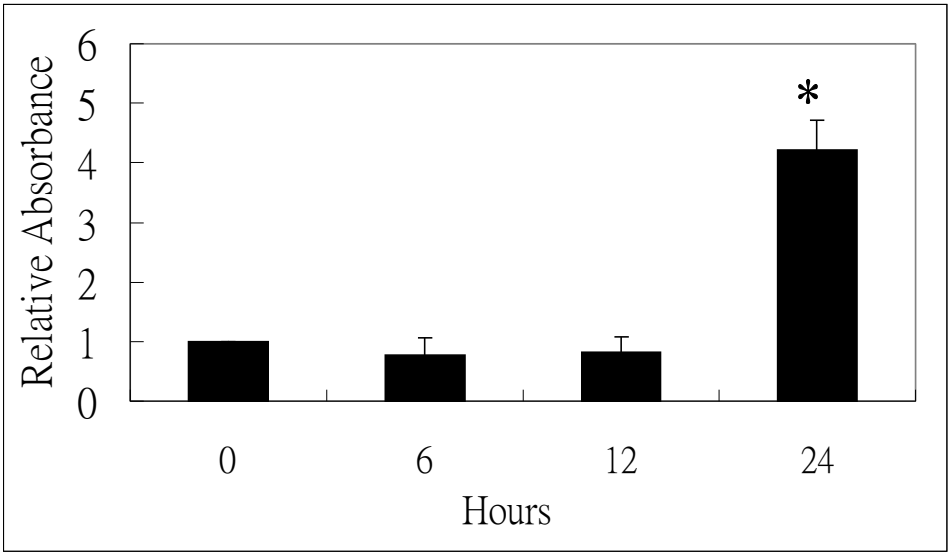


Fig.2A ATP activated caspase-3 in human granulosa-luteal cells. Human GLCs were incubated in a serum-free medium for 4 h prior to treatment, and were treated with increasing concentrations of ATP (10 μM, 100 μM, 1 mM, or 10 mM) for 24 h. Aliquots (30 μg) of cell lysate were subjected to 10% SDS-polyacrylamide gel electrophoresis under a reducing condition. A rabbit polyclonal antibody directed against the pro- (32 kD) and active (17 kD) caspase-3 was used, and the signal was visualized using an ECL system, followed by autoradiography.

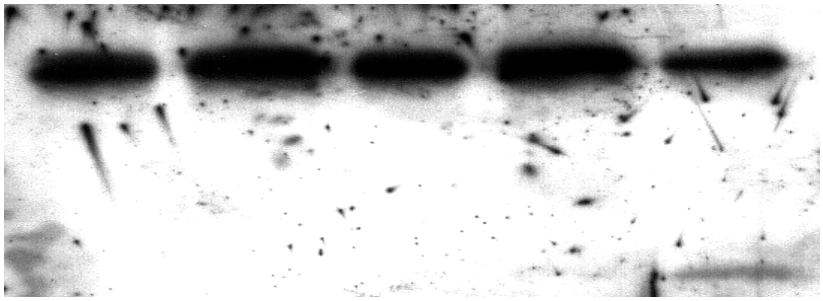


Fig.2B ATP activated caspase-3 in a time-dependent manner in human granulose-luteal cells. Human GLCs were incubated in a serum-free medium for 4 h prior to treatment, and were treated with 10 mM ATP for 6,12, or 24 hours. Aliquots (30 μg) of cell lysate were subjected to 10% SDS-polyacrylamide gel electrophoresis under a reducing condition. A rabbit polyclonal antibody directed against the pro- (32 kD) and active caspase-3 (17 kD) was used, and the signal was visualized using an ECL system, followed by autoradiography.

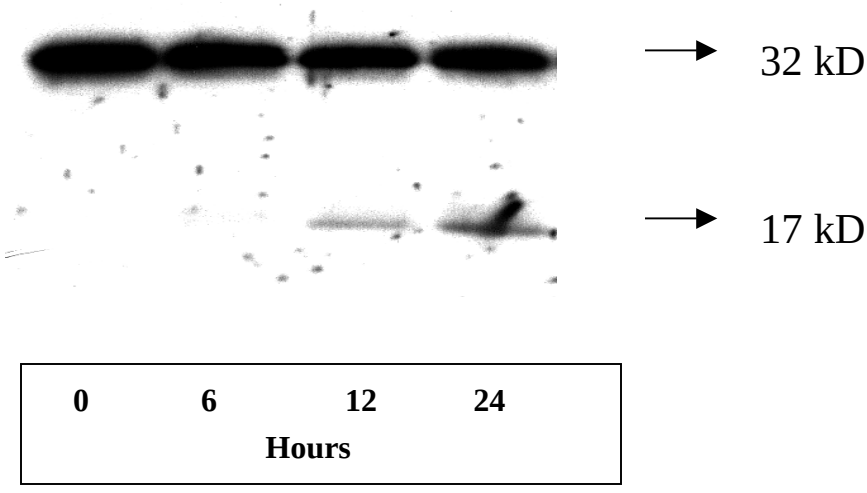


Fig.3A ATP induced cleavage of PARP in a dose-dependent manner in human granulosa-luteal cells. Human GLCs were incubated in a serum-free medium for 4 h prior to treatment, and were treated with increasing concentrations of ATP (10 μM, 100 μM, 1 mM or 10 mM) for 24 h. Aliquots (30 μg) of cell lysate were subjected to 10 % SDS-polyacrylamide gel electrophoresis under a reducing condition. A mouse monoclonal antibody directed against the PARP, which identified the 116 kD intact form as well as the 85 kD cleaved form, was used, and the signal was visualized using an ECL system, followed by autoradiography.

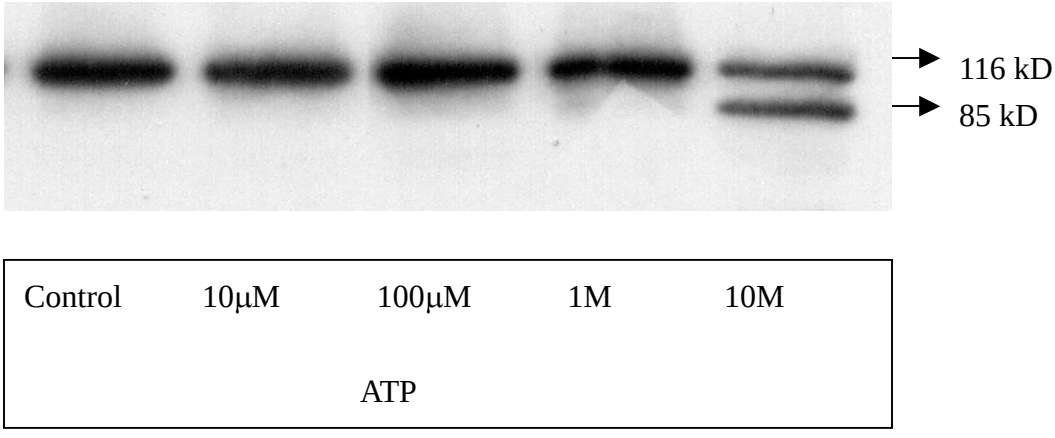


Fig.3B ATP induced cleavage of PARP in a dose-dependent manner in human granulosa-luteal cells. Human GLCs were incubated in a serum-free medium for 4 h prior to treatment, and were treated with 10 mM ATP for 6, 12, or 24 hours. Aliquots (30 μg) of cell lysate were subjected to 10% SDS-polyacrylamide gel electrophoresis under a reducing condition. A mouse monoclonal antibody directed against the PARP, which identified the 116 kD intact form as well as the 85 kD cleaved form, was used, and the signal was visualized using an ECL system, followed by autoradiography.

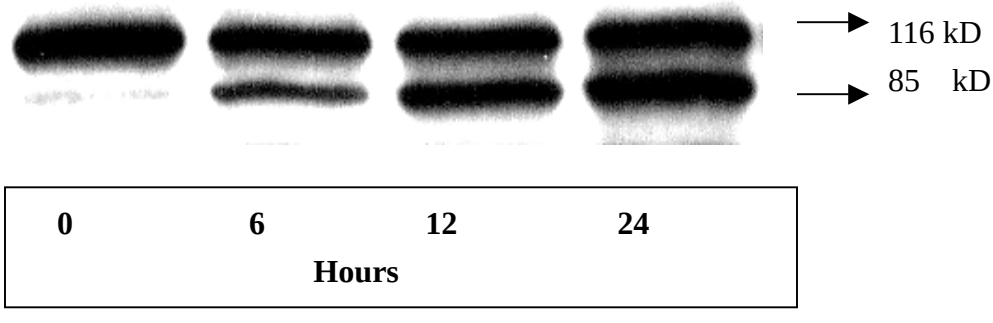
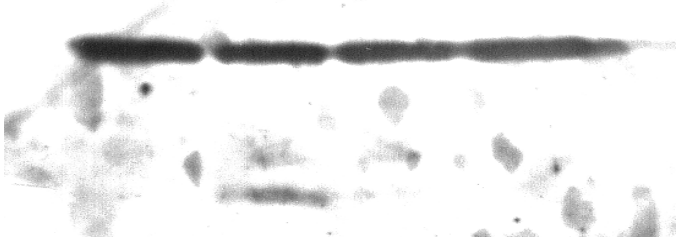
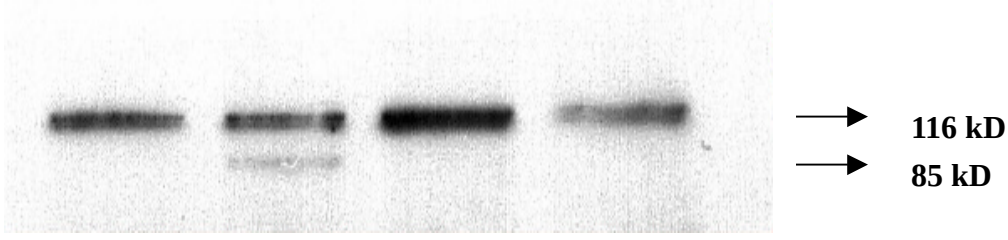


Fig.4A Effect of human chorionic gonadotropin (hCG) on protecting cells from apoptosis. Human GLCs were treated with 10 IU hCG in the presence of 10 mM ATP for 12 hours. Aliquots (30 g) of cell lysate were subjected to 10% SDS-polyacrylamide gel electrophoresis under a reducing condition. A rabbit polyclonal antibody directed against the pro- (32 kD) and active caspase-3 (17 kD) was used, and the signal was visualized using an ECL system, followed by autoradiography.



Control	ATP	hCG +ATP	ATP

Fig.4B Effect of human chorionic gonadotropin (hCG) on protecting cells from apoptosis. Human GLCs were treated with 10 IU hCG in the presence of 10 mM ATP for 12 hours. Aliquots (30 μ g) of cell lysate were subjected to 10% SDS-polyacrylamide gel electrophoresis under a reducing condition. A mouse monoclonal antibody directed against the PARP, which identified the 116 kD intact form as well as the 85 kD cleaved form, was used, and the signal was visualized using an ECL system, followed by autoradiography.



Control	ATP	hCG +ATP	ATP

成果自評：

本計劃延續本人在國外的研究成果，過去幾年，我的研究一直著眼於人類女性生殖細胞與內分泌系統關係的探討。我所研究的細胞包括顆粒細胞（granulosa-luteal cells）或卵巢上皮細胞（epithelium cells），迄今一共發表了 15 篇與女性生殖細胞，包括卵巢顆粒細胞、卵巢上皮細胞及子宮內膜細胞有關之研究，同時也積極參與國際會議並發表研究心得。先前我們曾發表腺嘌呤核甘三磷酸（ATP）會引發一連串的人體卵巢顆粒細胞內訊息傳遞系統，並且會調控人類絨毛膜刺性腺激素（human chorionic gonadotropin；hCG）對卵巢的作用。在此研究案中，我將國外所學之實驗技術及設計法則實際運用在國內的研究室，如我原本預期順利地在一年內完成研究計劃，同時也已完成論文發表方式的寫作，積極地在進行發表研究心得。本研究結果確實證明神經傳導物質在人體卵巢顆粒細胞可引發 caspase 系統的細胞內訊息傳遞路徑而引發細胞凋亡，同時在 ATP 所引起的細胞凋亡反應過程中，人類絨毛膜刺性腺激素（hCG）可以將其反應減弱。

去年我將本計劃案在執行期中所得到的部份成果，發表於在台灣台北圓山飯店所舉辦第三屆亞太地區生殖醫學會，從一百多篇參賽的論文中，獲得最有創意優秀論文獎第一名；很榮幸的在晚宴中接受呂副總統頒獎。今後我將更致力於指導國內研究生從事生殖醫學與內分泌系統之深入探討，藉此開發新的實驗計劃方向及拓展新視野，同時也培育國內的莘莘學子。最後非常感謝國科會自本人回國後這一年多來的支持與協助。