The in Vivo and in Vitro Stimulatory Effects of Cordycepin on Mouse Leydig Cell Steroidogenesis

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Cordycepin, a pure compound of Cordyceps sinensis (CS), is known as an adenosine analog. We have found that CS stimulated Leydig cell steroidogenesis. Here we investigated the in vivo and in vitro effects of cordycepin in primary mouse Leydig cell steroidogenesis. The results indicate that cordycepin increased the plasma testosterone concentration. Cordycepin also stimulated in vitro mouse Leydig cell testosterone production in dose- and time-dependent manners. We further observed that cordycepin regulated the mRNA expression of the A1, A2a, A2b, and A3 adenosine receptors in the mouse Leydig cells, and that antagonists of A1, A2a, and A3 suppressed testosterone production 20–50% testosterone production. Furthermore, Rp-cAMPS (cAMP antagonist) and Protein Kinase A (PKA) inhibitors (H89 and PKI) significantly decreased cordycepin-induced testosterone production, indicating that the PKA-cAMP signal pathway was activated by cordycepin through adenosine receptors. Moreover, cordycepin induced StAR protein expression, and H89 suppressed cordycepin-induced steroidogenic acute regulatory (StAR) protein expression. Conclusively, cordycepin associated with adenosine receptors to activate cAMP-PKA-StAR pathway and steroidogenesis in the mouse Leydig cells.

Key words: cordycepin; Leydig cell; adenosine; steroidogenesis; mouse

We have found that Cordyceps sinensis (CS) alone stimulated steroid production in both primary and tumor mouse Leydig cells.1,6 but it remains elusive which exact pure substance in CS stimulates steroidogenesis in Leydig cells. Many bioactive constituents from CS have been identified, including cordycepin,7 modified nucleosides,8 polysaccharides,9,10 and sterols, etc.11,12 Cordycepin (or 3’deoxyadenosine, an adenosine analog) is regarded as a signature constituent of Cordyceps species and a bioactive compound with many biological and pharmacological activities, including immunological regulation, anti-cancer effects, and anti-virus and anti-infection activities.13–17 Nevertheless, the role of cordycepin in the reproductive system is unknown in detail.

Adenosine and adenosine antagonists have been postulated to influence the male reproductive system.18,19 Moreover, mRNA expression of adenosine receptors in the rat testis has been confirmed,20 but the roles of adenosine in the testis remain unclear. Indeed, four different adenosine receptors that belong to the family of guanylyl nucleotide-binding protein (G-protein) G-protein coupled receptors (GPCRs) have been isolated in different tissues.21 These include the A1 and A3 adenosine receptors (A1-AR and A3-AR), which can interact with the inhibitory GTP-binding protein (Gi),22,23 and the A2a and A2b adenosine receptors (A2a-AR and A2b-AR), which interact with the stimulatory GTP-binding protein (Gs).24,25 They are typically coupled to the adenylylcyclase-cAMP signal-transduction pathway.

In the male reproductive system, gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the anterior pituitary to release luteinizing hormone (LH), which is transported to the testis to stimulate Leydig cells. LH is associated with the receptor to activate adenylyl cyclase through the G-protein. Activation of adenylyl cyclase results in the hydrolysis of ATP to cyclic AMP, which subsequently activates PKA,26 which results in protein phosphorylation and de novo protein synthesis, such as that of the StAR protein.27 The StAR protein can translocate free cholesterol from the outer to the inner mitochondrial membrane,28 where cholesterol is converted to pregnenolone29 by the P450 side-chain cleavage enzyme (P450scc). Pregnenolone is then transported to the smooth endoplasmic reticulum for further synthesis to testosterone, necessary steroid hormone for reproduction in males.30 Besides the PKA pathway, reports have identified that the PKC and MAPK signaling pathways are also involved in regulating steroidogenesis.31,32 Those three signaling pathways can also be regulated with the activation of adenosine receptors in different tissues.33–35 In fact, it has just been reported that adenosine receptors can be activated to induce the PKC pathway and then to induce rat adrenal cell steroidogenesis,35 but, the links among cordycepin, the adenosine receptor, the signal transduction pathway, and steroidogenesis in Leydig cell remain unknown. Hence the present study was designed to evaluate the in vivo and in vitro effects and the mechanism by which cordycepin regulates steroidogenesis in primary mouse Leydig cells.

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Materials and Methods

Chemicals. Cordycepin, dibutyryl AMP (dBcAMP), percoll gradient solution, 8-cyclonéptide-1,3-dipropylxanthine (DPCPX), 8- (3- chlorosteryl) caffeine (CSC), cycloheximide, 8-[(4-zyano phenyl)- carbamoylmethyl][oxy][phenyl]-1,3-di(4-propyl) xanthine (MRS 1754), Rp- Adenosine3’.5’-cyclic monophospho-thio-rhodiothiammonium salt (Rp-cAMPS), diethyl ether, N-[2-[(2-Bromomethylamino)methyl]ethoxy]-5-isoquinolinemethoxy-namido hydrochloride (B89) and 3-ethyl-5-benzyl-2-methyl-6-phenyl-ethylthienyl-6-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (MRS 1191) were purchased from Sigma (St. Louis, MO). PD98059 was from Tocris Cookson (Ellisville, MO). Staurosporine was from Calbiochem NovoBiochem (La Jolla, CA). M199 medium and fetal bovine serum (FBS) were from Gibco (Grand Island, NY). PKI (14–22 amide) was from Mallinckrodt Baker (Paris, Kentucky). Antibody against β-actin was from Cell Signaling (Beverly, MA). Antibody against StAR was the generous gift of Dr. Strauss Timothy (University of Pennsylvania Medical Center, Philadelphia, PA). Collagenase Type II was from Worthington Biochemical (Lakewood, NJ). Trizol reagent was from Invitrogen (Carlsbad, CA). dNTP and MMLV reverse transcriptase were from Promega (Madison, WI). Taq was from ABgene (Surrey, UK). [3H] testosterone, used in radioimmunoassay, was from DuPont-New England Nuclear (Boston, MA). Antiserum to testosterone was the kind gift of Dr. Paulus S. Wang (National Yang Ming University, Taipei, Taiwan).

Animals. Male B6 (C57BL/6N Crj) mice, 5–7 weeks old were purchased from the National Cheng Kung University Animal Center (Tainan, Taiwan). B6 mice should be less than 8 weeks old for experiments to exclude exposure of Leydig cells to LH. All the animals was housed in groups of four in 29 x 18 x 13 cm polyethylene cages. The animal room was maintained at 22–24°C (120 cycles/min) at 37°C the plasma. The plasma samples were stored at 29°C until assay for testosterone radioimmunoassay.36) The supernatant was poured into 2 mL of scintillation fluid and samples were counted in a β-counter for 2 min.

Immunoblot analysis. Immunoblot analysis was performed as previously described.35 In brief, 20 μg of proteins was solubilized in 1 x SDS sample buffer and loaded on a 12.5% SDS–PAGE minigel (Mini-Protein II System; Bio-Rad, Richmond, CA). Electrophoresis was performed at 100V for 100 min using standard SDS–PAGE running buffer. The proteins were transferred to polyvinylidene difluoride membranes (PVDF) (Bio-Rad) at 80 mA for 1 h in transfer buffer. The PVDF membrane with transferred protein was incubated in blocking buffer at room temperature for 1 h, and then incubated in fresh blocking buffer containing the primary antibody for 16–18 h at 4°C. After washing 3 times with PBS containing 0.5% Tween-20 for 30 min, the signal was detected with horseradish peroxidase-conjugated secondary antibody (Amersham, Piscataway, NJ) and visualized with Renaissance Chemiluminescence Reagent kit, as described by the manufacturer (NER: DuPont, Boston, MA). Proteins of interest were quantitated by a computer-assisted image analysis system (Quantity One, Huntington Station, NY). The amount of β-actin (43 kDa) in each lane was measured as a control to correct for expression of the StAR protein (30 kDa).

Isolation of total RNA and reverse-transcription polymerase chain reaction. Total RNA was isolated with Trizol Reagent (Carlsbad, CA) following the manufacturer’s instructions. Reverse transcription was performed in a mixture containing 5 μm random primer, 200 μm dNTP, 2 U/μL of MMLV reverse transcriptase, and 5 μL mRNA as template. The corresponding buffer was used at 42°C for 90 min followed by 95°C for 10 min. Polymerase chain reaction was performed in a mixture containing 2 μL of 10 × PCR buffer, 0.4 μL of 10 mm dNTP, 0.4 μL of 20 mm specific forward and reverse primers (the primer sequence and corresponding sequences of specific genes are listed in Table 1), 14.7 μL of dH2O, 0.1 μL 0.5 U Taq, and 2 μL of RT product as template for each reaction. The thermo-controlling program was set up as follows: 95°C for 30 s (denaturation), 55°C for 30 s (annealing), 72°C for 30 s (elongation), and another 5 min for each 9 cycles for 72°C. The entire mixture was subjected to 30 cycles for the amplification of L19 and StAR, 32 cycles for A1-AR, A2a-AR and A2b-AR, and 40 cycles for A3-AR. The PCR product was then separated on a 1.5% agarose gel at 120 V for 30 min in 1 × TBE buffer (0.09 m Tris, 0.09 m...
Boric acid, 0.001% EDTA, pH 8.0). The gel was stained with ethidium bromide for 10 min and destained with water. The gel image was captured using the Labwork Imager System (Digital CCD Camera, Hamamatsu Photonics System, Bridgewater, NJ).

Statistical analysis. Each data point in the figures represents the mean ± SEM (standard error of mean) of testosterone production in three separate experiments with at least duplicates of each treatment. StAR protein integrated optical density in percentage for three experiments. Statistically significant differences between treatments and controls were determined by one-way ANOVA followed by Least Significant Difference (LSD). Statistical significance was set at \( p < 0.05 \).

Results

In vivo stimulatory effects of cordycepin on testis weights and plasma testosterone concentration in mice

In the present experiment, immature B6 mice were used to determine the in vivo effects of cordycepin on reproductive functions. The results indicate that 20 mg/kg of cordycepin did not have any in vivo effect (data not shown). Also, 40 mg/kg of cordycepin had no effect on the body, prostate/seminal vesicle, or epididymis weights (Table 2) \( (p > 0.05) \). However, intraperitoneal administration of cordycepin (40 mg/kg) for 7 d significantly increased testis weights (by 0.1364 ± 0.003 g in the saline group and 0.1450 ± 0.003 g in the cordycepin-treated group; \( p < 0.05 \)) and the plasma testosterone concentration (2.88 ± 0.190 pg/µL in the saline group and 10.97 ± 2.313 pg/µL in the cordycepin-treated group, \( p < 0.01 \)) in the mice (Table 2).

Stimulatory effects of cordycepin on steroidogenesis in mouse Leydig cells

To verify that cordycepin directly stimulates testosterone production, purified primary mouse Leydig cells were treated with various concentrations of cordycepin.

Table 1. Sequence of Primers Used in Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>PCR size</th>
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</thead>
<tbody>
<tr>
<td>L19</td>
<td>F GAA ATC GCC AAT GCC AAC TC</td>
<td>405 bp</td>
</tr>
<tr>
<td></td>
<td>R TCT TAG ACC TGC GAG CCT CA</td>
<td></td>
</tr>
<tr>
<td>STAR</td>
<td>F ATG TTC TCT CTC GCT ACG TTC AA</td>
<td>451 bp</td>
</tr>
<tr>
<td></td>
<td>R TGA CAT TTG GGT TCT ACT CT</td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>F CGG GAT CCT ACA TCT CGG CCT TCC AGG</td>
<td>219 bp</td>
</tr>
<tr>
<td></td>
<td>R GGA ATT CAG TAG TGC GTG GCC CCA ATG</td>
<td></td>
</tr>
<tr>
<td>A2a</td>
<td>F CGG GAT CCG TCC CGG ACC TAC ATC GT</td>
<td>177 bp</td>
</tr>
<tr>
<td></td>
<td>R GGA ATT CGG TGC TGT AGG CAG TGA T</td>
<td></td>
</tr>
<tr>
<td>A2b</td>
<td>F CGG GTA CCC CTC GAC TGC ATT ACA GA</td>
<td>216 bp</td>
</tr>
<tr>
<td></td>
<td>R CGG CGG AAA CTT TTA TAC CGT AGC</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>F CGG GAT CCC GTG CCG TGG TCA GTT TG</td>
<td>384 bp</td>
</tr>
<tr>
<td></td>
<td>R GGA ATT CGG AGG CGT AGA CAA TAG G</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Effects of Cordycepin on Body Weight, Reproductive Organ Weights, and Plasma Testosterone Concentrations in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Body weight (g)</th>
<th>Prostate/ Seminal vesicle (g)</th>
<th>Epididymis (g)</th>
<th>Testes (g)</th>
<th>Testosterone (pg/µL)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>Saline</td>
<td>28</td>
<td>18.24 ± 0.27</td>
<td>0.0561 ± 0.002</td>
<td>0.0440 ± 0.001</td>
<td>0.1364 ± 0.003</td>
<td>2.88 ± 0.190</td>
</tr>
<tr>
<td>Cordycepin</td>
<td>40</td>
<td>28</td>
<td>17.93 ± 0.23</td>
<td>0.0566 ± 0.003</td>
<td>0.0463 ± 0.002</td>
<td>0.1450 ± 0.003**</td>
<td>10.97 ± 2.313**</td>
</tr>
</tbody>
</table>

\( p \) value | 0.2744 | 0.9076 | 0.3090 | 0.0139 | 0.0017 |

B6 mice (28/group) received a single IP injection of saline or 40 mg/kg cordycepin each day at 8:00 AM for 7 d. Values represent the mean ± SEM. Significant differences between the saline and the cordycepin group are denoted as \( p < 0.05 \) or \( p < 0.01 \).

Transcriptional expression of adenosine subtype receptors in mouse Leydig cells

Cordycepin is known to be the analog of adenosine, and four different adenosine receptors have been
isolated, including the A1, A2a, A2b, and A3 adenosine receptors.\textsuperscript{21,38} It is possible that cordycepin acts through the adenosine receptors to initiate steroidogenesis in mouse Leydig cells. Hence, mRNA expression of adenosine subtype receptors in primary mouse Leydig cells was determined by RT-PCR in the present study. The results indicate that the A1, A2a, A2b, and A3 receptors were all detected in primary mouse Leydig cells (Fig. 2A, B, E, and F). A1 and A3 adenosine receptor mRNA expression was not affected by cordycepin ($p > 0.05$) (Fig. 2C and H). However, cordycepin at 1 and 5 mM upregulated the expression of A2a adenosine receptor mRNA by 7- and 3-fold respectively ($p < 0.05$) (Fig. 2D), whereas 1 and 5 mM cordycepin suppressed A2b adenosine receptor mRNA expression by 40% and 70% respectively ($p < 0.05$) (Fig. 2G).

Effects of adenosine subtype receptor antagonists on cordycepin-induced steroidogenesis in mouse Leydig cells

To determine which adenosine subtype receptor is activated by cordycepin, selective adenosine subtype receptor antagonists were used in co-treatment with cordycepin for 3h in primary mouse Leydig cells, and testosterone production was assayed by radioimmunoassay. The results indicated that A1-AR antagonist (DPCPX), A2a-AR antagonist (CSC), and A3-AR antagonist (MRS1191) can significantly suppress cordycepin-stimulated testosterone production, by 35% (Fig. 3A), 20% (Fig. 3B), and 50% (Fig. 3D) respectively ($p < 0.05$). However, A2b-AR antagonist (MRS 1754) did not suppress cordycepin-stimulated testosterone production ($p > 0.05$) (Fig. 3C). These results suggest that cordycepin acts through adenosine A1, A2a, and A3 receptors, but not A2b, in order of potency A3 $>$ A1 $>$ A2a to stimulate steroidogenesis in primary mouse Leydig cells.

Effects of cordycepin on cAMP-dependent signal pathway inducing steroidogenesis in mouse Leydig cells

To determine whether the cAMP-dependent signal pathway is involved in cordycepin-induced steroidogenesis, mouse Leydig cells were treated with dibutyryl-cAMP (100 μM) (dbcAMP, cAMP analog) and Rp-cAMPs (1 mM) (cAMP antagonist) in the presence and absence of the cordycepin (1 mM) for 3h, and testosterone production was then assayed. Cordycepin did not have any effect on dbcAMP-induced steroidogenesis ($p > 0.05$) (Fig. 4A). However, Rp-cAMPs suppressed cordycepin-stimulated testosterone production by 37% ($p < 0.05$) (Fig. 4B). These results suggest that the effect of cordycepin might activate the cAMP-dependent signaling pathway, stimulating mouse Leydig cell steroidogenesis.
Effects of cordycepin on PKA inducing steroidogenesis in mouse Leydig cells

Activation of the cAMP signaling pathway is usually accompanied by activation of PKA.39,40 Hence, primary mouse Leydig cells were treated with PKA inhibitors, H89 and PKI, in the presence and absence of cordycepin (1 mM) for 3 h, and testosterone production was assayed. Both H89 (50 μM) and PKI (4 μM) significantly inhibited cordycepin-stimulated testosterone production, by 100% and 97% respectively (p < 0.05) (Fig. 5A and B). This strongly suggests that cordycepin activated the PKA signal transduction pathway, stimulating mouse Leydig cell steroidogenesis.

Effects of cordycepin on de novo protein synthesis and StAR protein expression in mouse Leydig cells

It is well known that de novo protein synthesis and StAR protein expression are essential to steroidogenesis.43 Indeed, we have found that CS could induced de novo protein synthesis and StAR protein expression in mouse Leydig cells.5,6 In the present study, the possibilities de novo protein synthesis and StAR protein expression induced by cordycepin were also determined. Mouse Leydig cells were treated with cordycepin (1 mM) in the presence and the absence of cycloheximide (1 mM), a general protein synthesis inhibitor, for 3 h,
and testosterone production was assayed. Cycloheximide significantly suppressed cordycepin-stimulated testosterone production, by 100% \((p < 0.05)\) (Fig. 6A), which indicates that \textit{de novo} protein synthesis was necessary in cordycepin action on Leydig cell function.

The effects of cordycepin (1 and 5 mM for 3 h) on StAR protein and mRNA expression in mouse Leydig cells were further examined by Western blot and RT-PCR analysis. Cordycepin significantly stimulated StAR protein and mRNA expression in dose-dependent manners \((p < 0.05)\) (Fig. 6B and C). Cordycepin (5 mM) induced approximately a 2-fold increase in StAR protein expression as compared to control (Fig. 6D). Likewise, cordycepin (1 mM and 5 mM) induced 1.5- to 2-fold increases in StAR mRNA expression (Fig. 6E). This indicates that cordycepin induces \textit{de novo} protein synthesis and StAR protein expression in mouse Leydig cell steroidogenesis.

**Effects of cordycepin on StAR protein expression through activation of the PKA signal pathway in mouse Leydig cells**

To determine the involvement of the PKA signaling pathway in cordycepin-induced StAR protein expression, H89 (50 \(\mu\)M) was administrated to mouse Leydig cells with cordycepin (1 and 5 mM) for 3 h. StAR protein expression was induced by cordycepin at 5 mM (Fig. 7A), but expression of the StAR protein was significantly suppressed by H89 \((p < 0.05)\). Normalization of StAR protein expression was detected (Fig. 7B). H89 significantly reduced cordycepin-stimulated StAR protein expression up to 66% \((p < 0.05)\). This confirms that the cordycepin activates PKA signaling pathway, inducing expression of the StAR protein and increasing testosterone production in primary mouse Leydig cells.

**Discussion**

*Cordyceps sinensis*, a parasitic fungus on the larvae of Lepidoptera, is used as a Chinese medicine. It is of great importance to explore the major chemical constituents that direct the function of this herb. Cordycepin (3'-deoxyadenosine) was first reported as a metabolic product originally isolated from cultures of *C. militaris* and thereafter, it has been known as bioactive component of natural *Cordyceps sinensis* and cultured *Cordyceps*. Our study is the first to indicate the role of cordycepin as a bioactive metabolite of *Cordyceps* in the regulation of steroidogenesis in male reproductive tissue.

Many studies, including one of human male infertility, indicate that Leydig cell function loss and sperm production decreases are due to drops in the testosterone concentration in blood. Thus testosterone is essential to male reproduction. In fact, we have found that oral administration of *Cordyceps sinensis* and its fractions increased plasma testosterone levels in both immature and mature B6 mice. Here we explain that cordycepin stimulated immature B6 mice plasma testosterone production accompanied by increasing testis weight, which suggests that cordycepin is the bioactive pure material of *Cordyceps* regulating Leydig cell function.

It has been found that adenosine analogs can stimulate steroid production and adenylate cyclase activity in I-10 mouse Leydig tumor cells and rat adrenal cells, but the expression and characterization of adenosine recep-
tors in Leydig cells remains unknown. In the present study, the expression of four adenosine subtype receptor mRNAs, A1, A2a, A2b, and A3, in primary mouse Leydig cells were detected, the first time they were demonstrated in reproductive tissue. Reports have co-existing adenosine subtype receptors in different combinations in a number of cells, such as A1 and A2a in glomerular and mesangial cells,47,48 and A1, A2a, and A2b adenosine receptor49 in porcine coronary artery smooth muscle cells. Thus the co-expression of four adenosine subtype receptors in primary mouse Leydig cells in the present study is not unprecedented.

It has been reported that the growth of B16-B16 mouse melanoma (B16-B16) cells was inhibited by cordycepin, and that its effect was antagonized by MRS1191, a selective adenosine A3 receptor antagonist.16 In addition, radioligand binding assay indicated that B16-B16 cells express adenosine A3 receptor and that cordycepin binds to these receptors.16 In the present study, we found that A1, A2a, and A3, but not A2b, adenosine receptor selective antagonists suppressed testosterone production induced by cordycepin in primary mouse Leydig cells of varying potency (A3 > A1 > A2a). In addition, cordycepin had different effects on mRNA expression among these four adenosine subtype receptors in primary mouse Leydig cells. Cordycepin upregulated A2a adenosine receptor mRNA expression, but suppressed A2b adenosine receptor mRNA expression, and had no effects on the A1 and

![Fig. 6. Effects of Cordycepin on StAR Protein and StAR mRNA Expression in Mouse Leydig Cells.](image)

Cells were treated with cordycepin (1 and 5 mM) in the presence and the absence of cycloheximide (1 mM) for 3 h. Media were collected and assayed for testosterone production by RIA (A). Blots illustrate the expression of StAR protein (B) and StAR mRNA (C) in response to cordycepin (1 and 5 mM) respectively for 3 h; (con = control). Integrated absorbance of StAR protein expression with β-actin normalization and StAR mRNA expression with L19 normalization is demonstrated in D and E respectively. Each data point in the figure represents the mean ± SEM for three independent experiments. Different numbers above the bars in A indicate that groups differ significantly from each other (p < 0.05). An asterisk above the column in D and E indicates that groups significantly differ from control (p < 0.05).

![Fig. 7. Effects of H89, a PKA Inhibitor, on Cordycepin-Induced StAR Protein Expression in Mouse Leydig Cells.](image)

Cells were treated with cordycepin (1 and 5 mM) in the presence or absence of H89 (50 μM) for 3 h. Total protein was extracted and Western blot detecting StAR protein expression was conducted, and illustrated in A. Integrated absorbance of StAR protein expression after normalization with β-actin shown in B. Each data point in the figure represents the mean ± SEM for three independent experiments. An asterisk above the column indicates that the group differ significantly from the other groups (p < 0.05), (con = control).
A2b receptor expression respectively, and that ischemia or TNF-alpha upregulated adenosine A1 and A2b receptor expression respectively. Thus it is possible that cordycepin induces A2a adenosine receptor expression to enhance Leydig cell steroidogenesis, consistently with other studies. More studies are needed to clarify the relationships.

Expression of more than one type of adenosine receptor on the same cell may allow the common agonist of adenosine to activate multiple signaling pathways. The stimulation of adenosine subtype receptors is associated with changes in second messenger cAMP levels with the involvement of the PKA, PKC or MAPK pathways. Evidence has indicated that cordycepin upregulates the cellular level of cAMP and cGMP in collagen-stimulated platelets. In fact, our data indicate that cordycepin activates the cAMP-dependent signaling through PKA, but not the PKC or MAPK pathway, to induce testosterone production in primary mouse Leydig cells that in cordycepin antagonist and PKA inhibitors significantly diminished cordycepin-stimulated testosterone production to almost 100%, whereas the PKC and MAPK inhibitors did not have any effect. This is comparable to the phenomenon that cAMP-PKA signal transduction is the major pathway activated by trophic hormone to induce steroidogenesis in Leydig cells. However, we cannot explain why the A1 and A3 receptors were also activated by cordycepin, which involves G protein coupling inactivating cAMP-PKA signal transduction pathway, to stimulate mouse Leydig cell steroidogenesis in the present study. More studies are necessary to uncover the truth as to discrepancy.

It is well established that StAR protein is essential for steroidogenesis, in which the expression of StAR protein is activated through cAMP-PKA signal transduction, inducing steroidogenesis. Our data indicate that cordycepin significantly upregulated the expression of StAR mRNA and StAR protein in primary mouse Leydig cells. That PKA inhibitor suppressed StAR protein expression almost 100% further suggests that the cAMP-PKA-StAR protein pathway is the main route activated by cordycepin, stimulating mouse Leydig cell steroidogenesis. In fact, this is consistent with our previous finding for CS-stimulated steroidogenesis in mouse Leydig cells.

In conclusion, cordycepin is one of the active components in CS that activate the cAMP-PKA signal transduction pathway to induce StAR protein expression and then to enhance testosterone production in primary mouse Leydig cells, possibly by exerting its effect through adenosine subtype receptors A1, A2a, and A3.

Acknowledgments

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References

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