Adenosine Triphosphate Induces Activation of Caspase-3 in Apoptosis of Human Granulosa-luteal Cells

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摘要

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 human granulosa-luteal cells (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. To examine the dose-response relationship, hGLCs were treated with increasing concentrations of ATP (10 .MU.M, 100 .MU.M, 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. (Author abst.).