

Expression and regulation of P2U-purinoceptor in human granulosa-luteal cells

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摘要

Abstract

The P2U purinoceptor (P2UR) has been identified pharmacologically in the ovary. However, the expression and regulation of the P2UR messenger RNA (mRNA) in human ovarian cells are still poorly characterized. The present study was designed to examine the expression and regulation of the P2UR in human granulosa-luteal cells (hGLCs) by RT-PCR and Northern blot analysis. A PCR product corresponding to the expected 599-bp P2UR complementary DNA was obtained from hGLCs. Molecular cloning and sequencing of the PCR product revealed an identical sequence to the reported P2UR complementary DNA. Two mRNA transcripts of 2.0 kb and 4.6 kb were identified in hGLCs using Northern blot analysis. The expression of the P2UR mRNA was down-regulated by human CG in a dose- and time-dependent manner. Treatment with 8-bromo-cAMP and forskolin also attenuated P2UR mRNA levels. Calcium signaling following the activation of the P2UR in single hGLCs was studied using microspectrofluorimetry. It revealed that, like ATP, uridine triphosphate (UTP) also induced cytosolic calcium mobilization in a dose-dependent manner. These results demonstrate for the first time that the P2UR mRNA is expressed in hGLCs and that P2UR mRNA is regulated by human CG, cAMP, and forskolin. The P2UR expressed in hGLCs functional because activation of the P2UR by ATP or UTP resulted in rapid and transient mobilization of cytosolic calcium at the single cell level. These findings further support a potential role of this neurotransmitter receptor in the human ovary.

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