Extracellular ATP Stimulates Steroidogenesis in Bovine Adrenocortical Fasciculata Cells via P₂ PURINOCEPTORS

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ABSTRACT—Effects of ATP and other purine derivatives on steroidogenesis in primary cultured bovine adrenocortical fasciculata cells were examined. At concentrations higher than 1 μM, ATP showed a potent stimulative effect on the cortisol production of the cells. The potency order of the steroidogenic effect of the tested purine derivatives was ATP > ADP >> adenosine >> AMP. α,β-Methylene ATP had no stimulative effect on the steroidogenesis at concentrations as high as 1 mM. Theophylline did not antagonize the steroidogenic effect of ATP. These results suggest that bovine adrenocortical fasciculata cells possess the P₂y purinoceptors that are linked to steroidogenesis.

Recently, many researchers have proposed that extracellular ATP is involved in the regulation of many biological functions; and the plasma concentration of ATP is elevated above 10 mM under stressful conditions (1). Because glucocorticoid from the adrenal cortex plays an important role in adapting the body to stressors, the effect of extracellular ATP on steroidogenesis in bovine adrenocortical fasciculata cells was investigated.

The isolated bovine adrenocortical fasciculata cells were prepared aseptically by the use of collagenase (Cooper Biomedical) and deoxyribonuclease (Sigma Chemical Co.). The isolated cells were cultured as previously described in Ham's F-10 medium supplemented with sera and antibiotics (2). The 3-day primary cultured monolayer cells were incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 3 mg/ml bovine serum albumin and 1 mg/ml glucose for 1 hr at 37°C in a CO₂-incubator (5% CO₂ in air) in the presence or absence of the test reagents (2). Glucocorticoid in the incubation medium was determined fluorometrically using cortisol as the standard (3).

Extracellular ATP had a stimulative effect on cortisol production at concentrations higher than 1 μM, and the maximum response was obtained at 100 μM (Fig. 1). The result indicated that bovine adrenocortical cells have purinoceptors that are linked to steroidogenesis, and the physiological concentration of ATP influences adrenocortical function. To estimate the receptor subtype, the effects of other purine derivatives on steroidogenesis were examined. At concentrations tested, up to 1 mM, ADP, AMP, and adenosine also stimulated steroidogenesis. However, the potency order of the effect was ATP > ADP >> adenosine >> AMP. α,β-Methylene ATP did not stimulate steroidogenesis at concentra-
tions up to 1 mM (Fig. 1). Theophylline could not inhibit the steroidogenic effect of ATP (data not shown).

The purinoceptors are known to have two receptor types, P1 and P2. It has been established that adenosine is a P1 purinoceptor agonist and ATP is a P2 purinoceptor agonist (1). From our results, in bovine adrenocortical cells, it is considered that there are two possibilities in the steroidogenic effect of ATP. One is that the effect of ATP appears via P2 purinoceptors. The other possibility is that ATP is metabolized to adenosine by exonucleotidases during the incubation period and the steroidogenic effect is performed by the metabolite adenosine, via P1 purinoceptors. However, the latter possibility can be abandoned because the steroidogenic potency order was ATP >>> adenosine, and theophylline, which is specific P1 purinoceptor antagonist (4), did not antagonize the effect of ATP. Thus it seems appropriate to assume the former possibility: the steroidogenic effect of extracellular ATP is the consequence of its action on P2 purinoceptors.

The P2 purinoceptors can be separated into at least two subtypes; namely, P2X and P2Y (1). Burnstock and Kennedy separated these receptors on the basis of the rank order of agonists' potencies of ATP analogues (5). The P2Y purinoceptor is stimulated in the potency order of ATP >> α,β-methylene ATP. At the P2X receptor, the agonist potency order of α,β-methylene ATP >> ATP is proposed. Therefore, the receptor subtype of bovine adrenocortical P2 purinoceptors might be P2Y.

In the present study, we suggested the existence of P2Y purinoceptors which induce steroidogenesis in bovine adrenocortical fasciculata cells. However, further investigations are required to establish the purinergic receptor type in bovine adrenocortical fasciculata cells and its physiological role in stress.
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