Volume 243, number 2, 333–336 FEB 06744 January 1989

Extracellular adenosine triphosphate activates phospholipase C and mobilizes intracellular calcium in primary cultures of sheep anterior pituitary cells

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Received 7 November 1988

In primary cultures of sheep anterior pituitary cells extracellular ATP (ED_{50} 0.4–0.8 μM) stimulated efflux of $^{45}Ca^{2+}$ from a slow-turnover intracellular pool. ADP was also effective whereas AMP and adenosine were not. The ATP effect was not due to cell permeabilization as 100 μM ATP did not elicit efflux of 2-deoxy[^{3}H]glucose metabolites. This $^{45}Ca^{2+}$ mobilization may be mediated by inositol trisphosphate, since ATP (ED_{50} 1 μM) stimulated inositol phosphate generation. These results demonstrate P_2-purinoceptors in sheep anterior pituitary cells which are coupled to phospholipase C activation and intracellular Ca^{2+} mobilization, and raise the possibility of a regulatory role for extracellular ATP in the anterior pituitary.

ATP; Ca^{2+} efflux; Purinoreceptor; Inositol phosphate; (Anterior pituitary)

1. INTRODUCTION

Extracellular ATP has biological activity at low micromolar concentrations in several cell types and is released in substantial quantities as a component of the secretory vesicles of platelets, neurons and adrenal medullary cells [1]. Based on these findings, a regulatory role for extracellular ATP has been postulated [1]. At low concentrations (<50 μM) ATP is thought to act by binding to specific receptors, designated P_2-purinoceptors, several subtypes of which have been delineated pharmacologically [2]. P_2-purinoceptors are activated by adenine nucleotides with the potency order ATP > ADP > AMP > adenosine, distinguishing them from P_1-purinoceptors which have the potency order adenosine > AMP > ADP ATP [1].

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At higher concentrations (>50 μM) ATP is able to permeabilize the plasma membranes of rat mast cells [3] and several mouse cell lines [4,5] to molecules of $M_r < 1000$. Free ATP (ATP^{4-}) rather than ATP complexed to cations is the form responsible for this effect [5,6] and high concentrations of total ATP (50–100 μM) are required for permeabilization when millimolar concentrations of Ca^{2+} and Mg^{2+} are present, as in typical physiological buffers.

Extracellular ATP at low micromolar concentrations is reported to stimulate inositol 1,4,5-trisphosphate production and intracellular Ca^{2+} mobilization in hepatocytes, endothelial cells, myocytes and Ehrlich ascites tumor cells [7–10]. These results suggest that P_2-purinoceptors are coupled to a signal-transduction pathway involving phospholipase C activation and Ca^{2+} mobilization.

We report here that extracellular ATP at submicromolar concentrations stimulates $^{45}Ca^{2+}$ efflux and inositol phosphate production in primary cultures of sheep anterior pituitary cells.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

2. MATERIALS AND METHODS

Anterior pituitaries from 6–12-month-old castrated male sheep were dispersed as described for chicken pituitaries [11] and used after 48 h of culture. For determination of $^{45}Ca^{2+}$ efflux cells were loaded with $^{45}Ca^{2+}$ and washed extensively as in [11]. Efflux buffer was removed for counting and replaced repeatedly at the indicated times. To determine efflux of 2-deoxy[^{3}H]glucose metabolites, attached pituitary cells were loaded with 2-deoxy[^{3}H]glucose (0.4 μCi/ml) in glucose-free physiological buffer (buffer A in [11]) for 1 h at 37°C. After extensive washing 2 ml buffer A was added to each well and 0.5-ml aliquots were counted at the indicated times. Cellular $^{45}Ca^{2+}$ and 2-deoxy[^{3}H]glucose were measured after solubilizing with 0.5% SDS.

Inositol phosphate production was measured as described [11] except that after elution of glycerophosphoinositol with 5 mM disodium tetraborate/60 mM sodium formate, total inositol phosphates were eluted in a single step using 0.1 M formic acid/1.0 M ammonium formate. All experiments were conducted at least twice. Each point and error bar shown represents the mean and SE of triplicate determinations.

$^{45}CaCl_2$ was obtained from New England Nuclear (Dreieich, FRG). 2-Deoxy[^{3}H]glucose and *myo*-[2-^{3}H]inositol were purchased from The Radiochemical Centre (Amersham, England). All other chemicals were from Sigma (St. Louis, MO). Staphylococcal α-toxin was a kind gift from Dr Sucharit Bhakdi (Department of Medical Microbiology, Justus-Liebig University, Giessen, FRG).

3. RESULTS AND DISCUSSION

Extracellular ATP stimulated efflux of $^{45}Ca^{2+}$ from a slow-turnover intracellular pool in primary cultures of sheep anterior pituitary cells (fig.1). The onset of ATP-induced $^{45}Ca^{2+}$ efflux occurred within 60 s (fig.1) and the ED_{50} was 0.4–0.8 μM total ATP in the presence of 1 mM Ca^{2+} and 1 mM Mg^{2+} (fig.2). The extent of $^{45}Ca^{2+}$ mobilized reached 40% of total cellular $^{45}Ca^{2+}$ after 6 min of stimulation (fig.1), indicating that a considerable proportion of the cells were stimulated by ATP. In Mg^{2+}-free medium the potency of ATP was increased (ED_{50} 0.1 μM; not shown) suggesting that free ATP^{4-} is the active species. ADP (100 μM) was also effective in stimulating $^{45}Ca^{2+}$ efflux whereas AMP (100 μM) and adenosine (100 μM) were both inactive (fig.2). The concentration dependence and nucleotide specificity of these effects are compatible with an action through the binding of ATP to P_2-purinoceptors [1]. These receptors are found in several mammalian tissues [1], but have not been reported in the anterior pituitary.

ATP^{4-} is able to permeabilize certain cell types

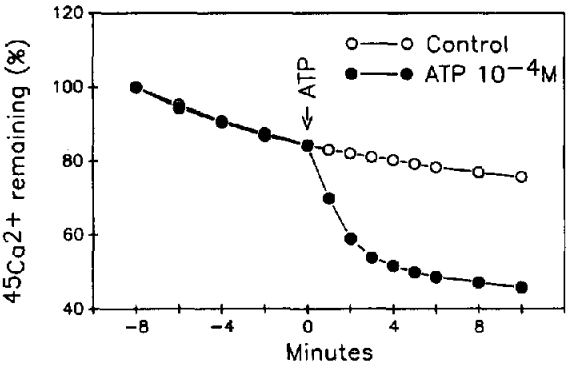

Fig.1. Time course of ATP-induced $^{45}Ca^{2+}$ efflux. Results are expressed as percent of cellular $^{45}Ca^{2+}$ present at $t = -8$ min. ATP (100 μM) was added at $t = 0$.

[3–5] and since this could result in $^{45}Ca^{2+}$ efflux, we examined the effect of ATP on efflux of plasma-membrane impermeant phosphorylated metabolites of 2-deoxy[^{3}H]glucose as an index of permeabilization. ATP (100 μM) caused no detectable permeabilization in contrast to the pore-forming cytolysin staphylococcal α-toxin (fig.3). This result excludes cell membrane permeabilization as a cause of the observed $^{45}Ca^{2+}$ efflux. Our results differ from those of Andrews et al. who

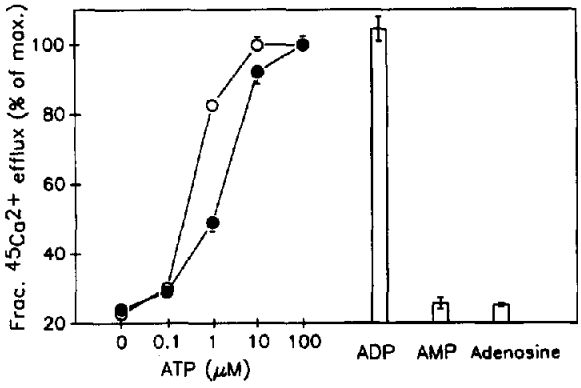

Fig.2. Dependence of $^{45}Ca^{2+}$ efflux on ATP concentration and effects of ADP, AMP and adenosine (all at 100 μM). Data shown are the fractional efflux of $^{45}Ca^{2+}$ which was calculated by dividing the amount of $^{45}Ca^{2+}$ effluxed during a 6 min period following addition of nucleotide by the amount of cellular $^{45}Ca^{2+}$ present at the beginning of the efflux period. Results are expressed as percent of maximum ATP-stimulated fractional efflux. Open circles and bars: primary sheep anterior pituitary cells. Filled circles: fibroblasts derived from sheep anterior pituitary cultures.

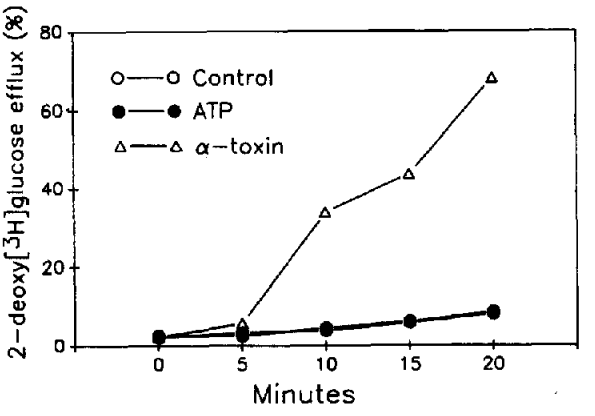

Fig.3. Efflux of 2-deoxy[^{3}H]glucose metabolites. ATP (100 μM) and α-toxin (3 μg/ml) were added at t = 0. Efflux of label is expressed as percent of total cellular label present at t = 0 min.

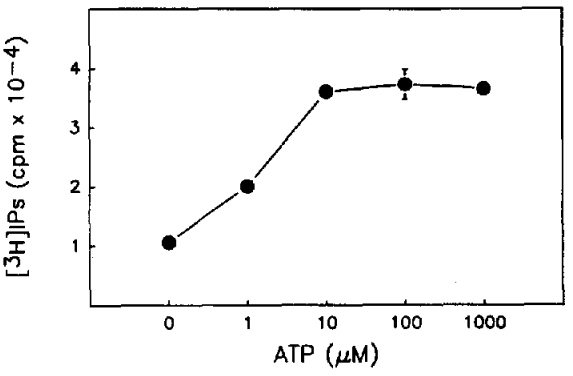

Fig.4. ATP-induced changes in cellular total [^{3}H]inositol phosphate (IPs) levels. Stimulation was for 10 min in the presence of Li^{2+} (10 mM).

reported that ATP permeabilized rat anterior pituitary cells to the dye trypan blue [12].

Coupling of P_2-purinoceptors to phospholipase C activation and intracellular Ca^{2+} mobilization has been demonstrated in several cell types [7–10]. In agreement with these results we found that ATP (ED_{50} 1 μM) stimulated an increase in total cellular inositol phosphates (fig.4). These findings suggest that the ATP-induced $^{45}Ca^{2+}$ mobilization we observed is mediated by inositol 1,4,5-trisphosphate generated by activation of phospholipase C.

Primary cultures of anterior pituitary cells consist initially of a mixture of endocrine cells and mesenchymal cells. Only the latter are able to divide in culture and as a result anterior pituitary cells cultured for more than 8 passages consist almost exclusively of mesenchymally derived cells with the morphology of fibroblasts. These cells also exhibit ATP-induced $^{45}Ca^{2+}$ mobilization (fig.2) and phospholipase C activation (not shown), suggesting that at least some of the ATP-responsive cells in primary pituitary cultures are fibroblasts or their progenator cells.

In order to ascertain what proportion of the ATP-responsive cells were cells which had proliferated during the 48 h culture period, cultures were treated with cytosine arabinoside to prevent cell division. Cultures treated with cytosine arabinoside at a concentration (10 μM) which inhibited [^{3}H]thymidine incorporation by more than 97% (not shown) showed no difference in ATP-induced $^{45}Ca^{2+}$ efflux compared with untreated cultures (not shown). This indicates that the ATP-responsive cells present in primary pituitary cultures are cells which are present in the pituitary in vivo.

The ability of ATP to stimulate inositol phosphate generation and intracellular Ca^{2+} mobilization in anterior pituitary cells raises the possibility of a physiological role for ATP. Clearly, the effects of ATP on pituitary hormone secretion and the localisation of P_2-purinoceptors to specific cell types requires investigation. The possibility that ATP has a non-endocrine role in the pituitary should also be entertained. One such possible role is the stimulation of vascular endothelial cells, which have been shown in other tissues to bear P_2-purinoceptors [1,10]. Furthermore, identification of adequate secretory sources of ATP (e.g. paracrine pituitary cell secretion or hypothalamic purinergic neuron secretion) is a prerequisite in assigning any physiological role to extracellular ATP.

Millimolar concentrations of ATP are often added to buffers used in permeabilized cell experiments including those employing anterior pituitary cells [13]. The presence of phospholipase C activating and Ca^{2+}-mobilizing ATP receptors could have unexpected and potentially misleading consequences where ATP is a component of the experimental media.

Acknowledgements: This work was supported by the South African Medical Research Council and the University of Cape Town.

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