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Undifferentiated HL60 cells respond to extracellular ATP and UTP by stimulating phospholipase C activation and exocytosis

Jane Stutchfield and Shamshad Cockcroft

Department of Physiology, Rockefeller Building, University College London, University Street, London WC1E 6JJ, UK

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We have recently characterised the presence of a Ca^{2+}-mobilising receptor for ATP which stimulates exocytosis in differentiated HL60 cells. Here we demonstrate that the undifferentiated HL60 cells also respond to extracellular ATP by stimulating an increase in inositol phosphates and exocytosis. Of the nucleotides (ATP, UTP, ITP, ATPγS, AppNHp, XTP, CTP, GTP, 8-Br-ATP and GTPγS) that were active in stimulating inositol phosphate formation, only UTP, ATP, ITP, ATPγS and AppNHp were active in stimulating secretion. On differentiation, the extent of secretion due to the purinergic agonists ATP, ITP, ATPγS and AppNHp remained unchanged whilst secretion due to UTP, a pyrimidine, was substantially increased. These results indicate that the effect of ATP and UTP may be mediated via separate purinergic and pyrimidinergic receptors, respectively.

ATP receptor; UTP receptor; Inositol phosphate; Secretion; Cytosol Ca^{2+}; Differentiation

1. INTRODUCTION

Differentiated HL60 cells and human neutrophils have recently been shown to respond to extracellular ATP and UTP [1–8]. Functional responses stimulated by ATP and UTP are exocytotic secretion [5,6] and superoxide generation [7,8]. The intracellular signalling pathways that may be responsible for mediating the functional responses are the activation of the inositol-lipid specific phospholipase C and phospholipase A_2. Thus it has been demonstrated that ATP and UTP can stimulate the formation of inositol phosphates [3,6], a rise in cytosol Ca^{2+} [4,5] and release of arachidonate [6].

The HL60 cells only acquire the receptors for fMetLeuPhe, C5a and leukotriene B_4 on differentiation and thus can only respond functionally to these agonists after the cells have been differentiated. Whilst the undifferentiated cells do not possess the components of the superoxide generating system [9] and thus cannot elicit a respiratory burst, the secretory mechanism is already present in the undifferentiated cells [10]. Thus undifferentiated cells secrete when Ca^{2+} and GTP analogues are introduced into streptolysin *O*-permeabilised cells [10].

Correspondence address: S. Cockcroft, Department of Physiology, Rockefeller Building, University College London, University Street, London WC1E 6JJ, UK

Abbreviations: ATPγS, adenosine 5′-[γ-thio]triphosphate; GTPγS, guanosine 5′-[γ-thio]triphosphate; AppNHp, adenosine 5′-[βγ-imido]triphosphate; PLC, phospholipase C

Dubyak and his colleagues have shown that the undifferentiated cell can respond to ATP and UTP by stimulating a rise in cytosol Ca^{2+} and the formation of inositol phosphates [3,4]. In this study we have investigated the ability of the undifferentiated cells to secrete when stimulated by extracellular ATP and UTP. We report that secretion due to ATP occurs to a similar extent to that seen in differentiated cells but secretion due to UTP is considerably less compared to differentiated cells suggesting that UTP and ATP stimulate secretion via separate receptors.

2. MATERIALS AND METHODS

All nucleotides were obtained from Boehringer-Mannheim, except XTP which was obtained from Sigma.

HL60 cells were grown in suspension culture and labelled with [^{3}H]inositol for 48 h as described previously [10]. When required, the cells were differentiated with 300 μM dibutyryl cyclic AMP towards a neutrophil-like cell as described previously [11].

HL60 cells (5×10^7) were washed 3 times in 8 ml of buffered salt solution (pH 7.2) comprising of 20 mM Hepes, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM LiCl, 1 mg/ml bovine albumin and 5.6 mM glucose and incubated for 30 min at 10^7 cells/ml at 37°C. The cells were treated with cytochalasin B (5 μg/ml final) and aliquots of 100 μl transferred to tubes containing an equal volume of buffer supplemented with nucleotides as indicated. After 10 min at 37°C, the cells were centrifuged for 5 min at 4°C at 1000 × *g* and 50 μl of supernatant was harvested for the determination of β-glucuronidase as described [6]. For the determination of inositol phosphates, the sedimented cells were quenched with chloroform/methanol and processed as in [6].

Intracellular Ca^{2+} was measured using Fura-2 as the indicator as described previously [5].

Data are expressed from individual experiments conducted on at least 3 different occassions.

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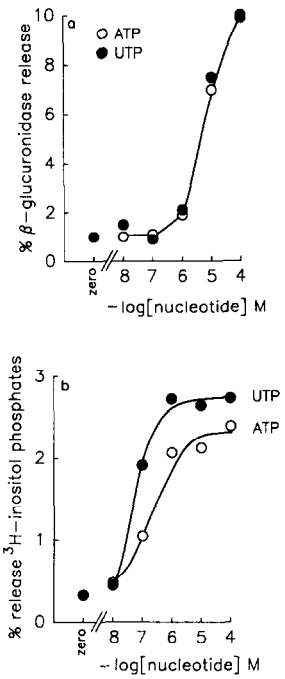

Fig.1. Effect of ATP and UTP on (a) β-glucuronidase secretion and (b) inositol phosphate production in undifferentiated HL60 cells. Open circles indicate incubations with ATP and closed circles indicate incubations with UTP.

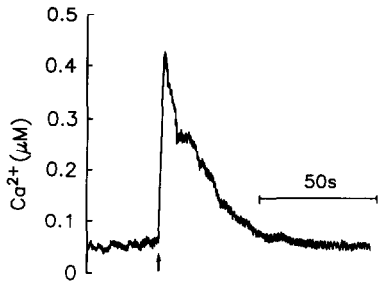

Fig.2. Changes in intracellular Ca^{2+} stimulated by 10 μM ATP in undifferentiated HL60 cells. Changes in cytosol Ca^{2+} concentration was determined using the intracellular indicator, Fura-2.

3. RESULTS AND DISCUSSION

Fig.1a and b illustrates the ability of extracellular ATP and UTP to stimulate secretion of β-glucuronidase and inositol phosphate production from undifferentiated HL60 cells. Fig.2 shows that ATP was also effective at causing a rise in cytosol Ca^{2+} from these cells as measured with Fura-2. UTP also stimulated a similar rise in cytosol Ca^{2+} (data not shown).

We next examined the effects of different nucleotides for stimulation of inositol phosphate formation and β-glucuronidase secretion from HL60 cells. The range of nucleotides tested was the same as that used in our earlier studies with differentiated HL60 cells. Nucleotides that were active in stimulating inositol phosphate formation were ATP, UTP, ITP, ATPγS, AppNHp, XTP, CTP, GTP, 8-Br-ATP and GTPγS which is identical to the range that is active in the differentiated HL60 cells [6]. Although all these nucleotides yielded a similar magnitude of inositol phosphates formation at 100 μM, the sensitivity of the responses at lower nucleotide concentrations differed with various nucleotides. Thus only ATP, UTP, ITP and ATPγS showed an increase in inositol phosphates at 1 μM.

Of all the nucleotides that were active in stimulating inositol phosphate formation, only ATP, UTP, ITP and ATPγS were able to promote secretion. These are the 4 nucleotides that were the most sensitive in stimulating inositol phosphate formation. Qualitatively, these observations are identical to that observed previously in differentiated HL60 cells [6].

However, one difference was observed between undifferentiated and differentiated cells. Whilst the extent of secretion in undifferentiated and differentiated cells with ATP, ITP and ATPγS is similar, the response to UTP is considerably enhanced upon differentiation (table 1). This increase in responsiveness with UTP upon differentiation only affects the secretory response but not the increase in inositol phosphates. As expected, fMetLeuPhe only stimulates secretion from differentiated cells (table 1).

Table 1

Comparison of secretory response in undifferentiated and dibutyryl cAMP differentiated HL60 cells

Agonist	Concentration (μM)	% β-Glucuronidase secretion	
		Undifferentiated HL60 cells	Differentiated HL60 cells
Control	0	1.3 ± 0.2 (6)	1.5 ± 0.1 (20)
ATP	100	10.3 ± 0.6 (6)	11.6 ± 1.1 (20)
UTP	100	9.1 ± 0.5 (4)	16.1 ± 2.1 (8)
ITP	100	6.0 ± 0.5 (3)	8.0 ± 1.1 (7)
ATPγS	100	6.0 ± 0.4 (3)	6.1 ± 0.9 (7)
fMetLeuPhe	0.1	1.5 ± 0.5 (6)	30.0 ± 2 (14)

Results are presented as % secretion and are means ± SE (number on individual experiments in parentheses)

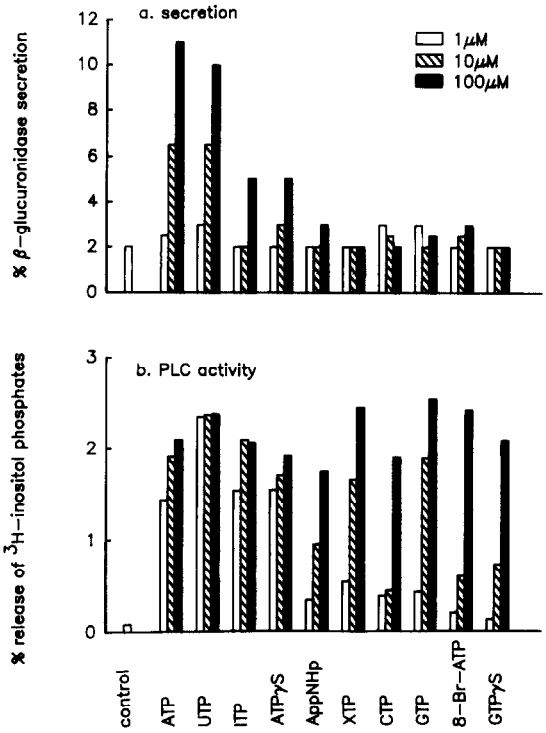

Fig.3. Effect of different nucleotides on (a) β-glucuronidase secretion and (b) inositol phosphate production in undifferentiated HL60 cells. Undifferentiated HL60 cells were incubated with the indicated nucleotides for 10 min. The bars which represent incubations in the presence of nucleotides are: open bars, 1 μM; hatched bars, 10 μM; and filled bars, 100 μM.

We have previously shown that the magnitude of the secretory response is not a function of the size of the Ca^{2+} signal. Thus the secretory response with fMetLeuPhe is greater than with ATP or UTP despite a similar increase in the level of cytosol Ca^{2+} [6]. This would indicate that upon differentiation, the enhancement of secretion by UTP is due to the activation of some additional intracellular signalling pathway(s) such as phospholipase A_2 or D which may modulate the extent of secretion.

Responses to both ATP and UTP have been observed in adrenal chromaffin cells [12], pituitary cells [13], fibroblasts [14], A431 cells [15], J774 mouse macrophage cell line [16], Ehrlich ascites cells [17], differentiated HL60 cells [5,7], human neutrophils [5] and perfused rat liver [18]. With the exception of adrenal chromaffin cells, in all the cell-types investigated, UTP was either more potent or equipotent with ATP. That the effect of UTP may not be mediated via the purinergic receptor but may occur via a separate pyrimidine receptor has been raised [7]. The observation that on differentiation of HL60 cells, only the response to UTP, a pyrimidine, is increased, but the response to ATP and other purinergic nucleotides remain unchanged would support the possibility that ATP and UTP may act on two different receptors.

In conclusion, undifferentiated HL60 cells, previously shown to respond by exocytosis only when permeabilised thus allowing access to Ca^{2+} and GTP analogues, are now shown to possess receptors for purines and pyrimidines which are coupled to phospholipase C leading to exocytosis. Comparative studies in undifferentiated and differentiated HL60 cells should provide some clues as to which signalling events control the magnitude of secretion on stimulation.

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REFERENCES

[1] Kuhns, D.B., Wright, D.G., Nath, J., Kaplan, S.S. and Basford, R.E. (1988) Lab. Invest. 58, 448-453.
[2] Ward, P.A., Cunningham, T.W., McCulloch, K.K. and Johnson, K.J. (1988) Lab. Invest. 58, 438-447.
[3] Dubyak, G.R., Cowen, D.S. and Meuller, L.M. (1988) J. Biol. Chem. 263, 18108-18117.
[4] Cowen, D.S., Lazarus, H.M., Shurin, S.B., Stoll, S.E. and Bubyak, G.R. (1989) J. Clin. Invest. 83, 1651-1660.
[5] Cockcroft, S. and Stutchfield, J. (1989) FEBS Lett. 245, 25-29.
[6] Cockcroft, S. and Stutchfield, J. (1989) Biochem. J. 263, 715-723.
[7] Seifert, R., Burde, R. and Schultz, G. (1989) Biochem. J. 259, 813-819.
[8] Kuroki, M. and Minakami, S. (1989) Biochem. Biophys. Res. Commun. 162, 377-380.
[9] Newburger, P.E., Speier, C., Borregaard, N., Walsh, C.E., Whitin, J.C. and Simons, E.R. (1984) J. Biol. Chem. 259, 3771-3776.
[10] Stutchfield, J. and Cockcroft, S. (1988) Biochem. J. 250, 375-382.
[11] Cockcroft, S. and Stutchfield, J. (1988) Biochem. J. 256, 343-350.
[12] Sasakawa, N., Nakaki, T., Yamamoto, S. and Kato, R. (1989) J. Neurochem. 52, 441-447.
[13] Davidson, J.S., Wakefield, I.K., Sohnius, U., Van der Merwe, P.A. and Millar, R.P. (1989) Endocrinology vol. 125.
[14] Fine, J., Cole, P. and Davidson, J.S. (1989) Biochem. J. 263, 371-376.
[15] Hosoi, K. and Edidin, M. (1989) Proc. Natl. Acad. Sci. USA 86, 4510-4514.
[16] Greenberg, S., Virgilio, F.D., Steinberg, T.H. and Silverstein, S.C. (1988) J. Biol. Chem. 263, 10337-10343.
[17] Dubyak, G.R. and De Young, M.B. (1985) J. Biol. Chem. 260, 10653-10661.
[18] Haussinger, D., Busshardt, E., Stehle, T., Stoll, B., Wettstein, M. and Gerok, W. (1988) Eur. J. Biochem. 178, 249-256.