Characterization of ATP receptor responsible for the activation of phospholipase A_2 and stimulation of prostaglandin E_2 production in thymic epithelial cells

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In TEA3A1 rat thymic epithelial cells, ATP stimulates prostaglandin E_2 (PGE_2) production through activation of phospholipase A_2 (PLA_2) enzymic activity. The stimulation of PGE_2 production tested with other nucleotides indicated the agonist potency of adenosine 5′-[γ-thio]triphosphate (ATP[S]) ≥ UTP > ATP, with ED_{50} of about 10 μM for ATP[S]. In TEA3A1 cells, cross-linking studies with ATP[^{35}S] revealed the presence of four cell-surface cross-linked bands of 42 kDa, 53 kDa, 83 kDa and 100 kDa in Triton X-100 extracts of TEA3A1 cells by fluorography. Guanosine 5′-[γ-thio]triphosphate specifically blocked the cross-linking of ATP[^{35}S] to the 53 kDa, 83 kDa and 100 kDa ATP-binding proteins, and inhibited the ATP[S]-mediated stimulation of PGE_2 production with an ED_{50} of about 25 μM. On the other hand, 2-methylthioadenosine triphosphate (2MeSATP) blocked ATP[^{35}S] cross-linking to the 42 kDa protein, but had no effect on ATP[S]-mediated stimulation of PGE_2 production. In a variant cell line, TEAvar1, derived from TEA3A1 cells that lost their response to ATP in the activation of PLA_2, the presence of 83 kDa ATP-binding protein was not detected. Results from our study suggest that ATP activates PLA_2 enzymic activity in TEA3A1 cells by binding to an atypical ATP receptor that has not been described previously.

INTRODUCTION

Arachidonic acid (AA) metabolites play an important role in thymocyte development in the thymus. For example, it has been shown that AA metabolites of the lipoxygenase pathway are involved in the interleukin-1-mediated induction of interleukin-2 receptor expression and in interleukin-2-mediated responses in T cells [1–5]. On the other hand, AA metabolites of the cyclooxygenase pathway have been shown to induce DNA fragmentation in thymocytes undergoing apoptosis [6,7] and to down-modulate interleukin-1 production [4,5]. The importance of AA metabolite production in the thymus is also evident from the observations made in lupus-prone autoimmune NZB/W mice. In young NZB/W mice, the level of prostaglandin E_2 (PGE_2) in the thymus is low. This low level of prostaglandin is related to thymocyte hyperplasia in the thymus cortex [8–10]. The injection of PGE_1 into NZB/W mice prevented the onset of this early-phase thymocyte hyperplasia in these animals [9]. Recent studies on fetal thymus organ culture also indicated that AA metabolites are important in the development, growth and function of T lymphoid cells in the thymus [11]. In thymus, AA metabolites are produced by thymic epithelial cells. Thus the elucidation of the regulatory mechanism of AA metabolism in thymic epithelial cells is of obvious importance. However, the physiological factor that regulates AA metabolism in thymic epithelial cells is not completely understood. In order to investigate this question, the rat thymic epithelial cell line TEA3A1 was used as a model system. The TEA3A1 cell line was established from primary culture of rat thymic epithelial cells [12]. TEA3A1 cells are not transformed, and retain functional properties of normal endocrine epithelial cells of the subcapsular and medullary region of the thymus. In the present paper, we provide evidence that AA metabolism is regulated by purinergic receptors in TEA3A1 thymic epithelial cells.

Extracellular ATP mediates a variety of biological responses, including phospholipase A_2 (PLA_2) activation, by binding to its cell surface receptors, collectively called P2 purinergic receptors [13–19]. There are five different subtypes of P2 receptors (x, y, z, t and u), pharmacologically characterized based on the differences in relative agonist potency of nucleotide analogues, biological functions and tissue distributions [15,19,20]. Biochemical characterization of ATP receptors has been carried out recently by using Scatchard analysis and photoaffinity labelling of ATP-binding proteins on plasma-membrane preparations from several tissues [21–23]. ATP-binding proteins with molecular masses of 62 kDa and 53 kDa and with K_d values in the nM range have been classified as P2x and P2y receptors respectively [21,23]. In addition, a 43 kDa ADP receptor, P2t, with K_d in the nM range has also been characterized in platelets by photoaffinity labelling [24]. Recently, cDNAs coding for 41 kDa P2y [25] and 42 kDa P2u [26] receptors have been cloned. Since the reported molecular mass for P2y is 53 kDa [23], the P2y receptor is likely to be glycosylated. Similarly, when cDNA for the P2u receptor was expressed in K562 human leukaemia and NG108-15 neuroblastoma–glioma hybrid cells, the P2u receptor was expressed as a 53 kDa glycosylated protein [26,27].

Here we report the characterization of the ATP receptor responsible for the activation of AA metabolism in TEA3A1 rat thymic epithelial cells. Results from our experiments indicate that the activation of PLA_2 enzymic activity is mediated by a

Abbreviations used: PGE_2, prostaglandin E_2; GTP[S], guanosine 5′-[γ-thio]triphosphate; ATP[S], adenosine 5′-[γ-thio]triphosphate; p[CH_2]ppA, adenosine 5′-[βγ-methylene]triphosphate; pp[CH_2]pA, adenosine 5′-[αβ-methylene]triphosphate; 2MeSATP, 2-methylthioadenosine triphosphate; PLA_2, phospholipase A_2; AA, arachidonic acid; DMP, dimethyl pimelimidate; DME, Dulbecco's Modified Eagle's Medium.

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83 kDa ATP receptor expressed on thymic epithelial cells. We have found that GTP and guanosine 5′-[γ-thio]triphosphate (GTP[S]) are potent antagonists of the 83 kDa ATP receptor and can block ATP-mediated stimulation of AA metabolism in TEA3A1 cells.

MATERIALS AND METHODS

Materials

Reagents used in the experiments were purchased from the following sources: high-glucose Dulbecco's Modified Eagle's Medium (DME) from GIBCO–BRL (Grand Island, NY, U.S.A.); Ca^{2+}-free WAJC404 medium from Kyokuto Pharmaceutical Co. (Tokyo, Japan); insulin, transferrin, dexamethasone, GTP[S], adenosine 5′-[γ-thio]triphosphate (ATP[S]) and iron-supplemented calf serum from Sigma Chemical Co. (St. Louis, MO, U.S.A.); UTP and adenosine 5′-[βγ-methylene]triphosphate (p[CH_2]ppA) from Boehringer (Mannheim, Germany); 5′-[αβ-methylene]triphosphate (pp[CH_2]pA) and 2-methylthioadenosine triphosphate (2MeSATP) from Research Biochemicals (Natick, MA, U.S.A.); dimethyl pimelimidate (DMP) from Pierce (Rockford, IL, U.S.A.); ATP[^{35}S] (1450 Ci/mmol), [^{3}H]PGE_2 (185 Ci/mmol) and [^{3}H]AA (76 Ci/mmol) from Du Pont–NEN Research Products (Boston, MA, U.S.A.).

Cell culture

TEA3A1 cells were grown to confluence in DW medium (1:1 mixture of DME and WAJC404A) supplemented with 10 μg/ml transferrin, 10 μg/ml insulin, 10 nM dexamethasone and 2% iron-supplemented calf serum in 6-well culture clusters (Costar, Cambridge, MA, U.S.A.) as previously described [28].

AA release assay

TEA3A1 cells cultured to confluence were prelabelled with 3 μCi/ml [^{3}H]AA for 6 h. AA release was assayed as previously described [29,30]. Briefly, the [^{3}H]AA released from the cells was extracted from the medium and analysed by TLC on silica gel G plates. TLC plates were developed in the organic phase of a mixture of ethyl acetate/heptane/acetic acid/water (38:12:17:33, by vol.), and spots migrating as the corresponding unlabelled AA standard were scraped and counted for radioactivity on a liquid-scintillation counter.

RIA for PGE_2

TEA3A1 cells cultured to confluence were washed with 3 × 2 ml of DME/well and with 1 ml of DME (containing 10 mg of fatty-acid-free BSA/ml)/well at 37 °C for 30 min. Immediately before the experiment, the cells were washed once with 2 ml of DME/well (37 °C) to remove BSA. All the experiments were carried out by incubating cells in 1 ml of DME/well in the presence or absence of indicated amounts of factors for 15 min at 37 °C unless stated otherwise. After 15 min incubation, the media were collected, centrifuged at 12000 *g* for 1 min, and the supernatants were assayed for PGE_2 by RIA. PGE_2 concentration in the media was determined by RIA as previously described [28].

Cross-linking of ATP[^{35}S] to TEA3A1 cells

ATP[^{35}S] binding was carried out as described above. The cells were incubated with 100 nM of ATP[^{35}S] (125 μCi/nmol) in the absence or in the presence of various doses (0–200 μM) of 2MeSATP, GTP[S] or ATP[S] for 3 h at room temperature. After washing twice with PBS, cross-linking was carried out by incubating the cells with PBS containing 10 mM of the cross-linking agent DMP for 10 min at room temperature as described previously [31]. After washing with PBS, the cells were harvested with a disposable cell scraper (Costar) in 1 ml of ice-cold PBS containing 500 μM phenylmethanesulphonyl fluoride, 25 μg/ml each of leupeptin and aprotinin and 2 mM EDTA. Samples were centrifuged at 700 *g* for 5 min. Supernatants were discarded, and the pellets were suspended into 25 μl of PBS containing 1% Triton X-100 and incubated for 15 min at room temperature. Samples were centrifuged for 5 min at 13000 *g*, and 20 μl of supernatant from each sample was mixed with 20 μl of 2 × Laemmli's sample buffer [32] containing 2% β-mercaptoethanol and boiled for 5 min. Electrophoresis of samples (75 μg of protein/lane) was carried out on SDS/10%-polyacrylamide slab gels as described by Laemmli [32] in a mini gel apparatus (Bio-Rad, Richmond, CA, U.S.A.). Fluorography was carried out by the method of Laskey and Mills [33].

RESULTS

Stimulation of AA metabolism in TEA3A1 cells by ATP

In TEA3A1 cells, PLA_2 is the rate-limiting enzyme that regulates AA metabolism [28–30,34]. Experiments were carried out to investigate the physiological factors that stimulate PLA_2 enzymic activity in TEA3A1 cells. For determining PLA_2 enzymic activity by using intact cells, the release of [^{3}H]AA from prelabelled TEA3A1 cells was measured. Among many of the physiological factors tested, we have found that ATP stimulated [^{3}H]AA release from TEA3A1 cells. Over a 3-fold increase in the stimulation of [^{3}H]AA release was observed in [^{3}H]AA-prelabelled cells treated with 50 μM ATP for 15 min. The major AA metabolite produced by TEA3A1 thymic epithelial cells is PGE_2 [28]. The treatment of cells with ATP had no effect on cyclooxygenase enzymic activity estimated by using intact cells (results not shown). Thus PGE_2 production was monitored by RIA in our experiments in order to assess the activation of PLA_2 enzymic activity in TEA3A1 cells. ATP-mediated stimulation of PGE_2 production in TEA3A1 cells was time- and dose-dependent. TEA3A1 cells were incubated with 20 μM ATP for various lengths of time (1.5–60 min) at 37 °C. As shown in Figure 1, a rapid stimulation of PGE_2 production took place within 1.5 min after addition of ATP in TEA3A1 cells. PGE_2 production reached a plateau after 5 min of incubation, where over a 12-fold stimulation of PGE_2 production was observed. Since different

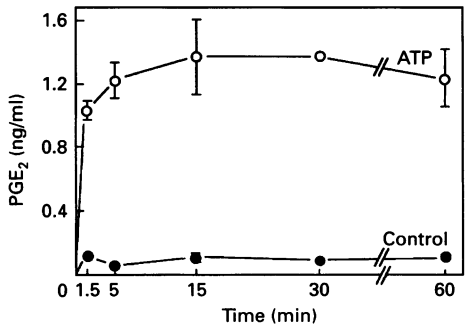

Figure 1 Time-dependent increase in PGE_2 production by ATP in TEA3A1 cells

Confluent TEA3A1 cells were treated for increasing lengths of time at 37 °C with 20 μM ATP. Media were collected after the incubation period and the level of PGE_2 produced by the cells was determined by RIA. Each point represents the mean ± S.D. ($n = 3$).

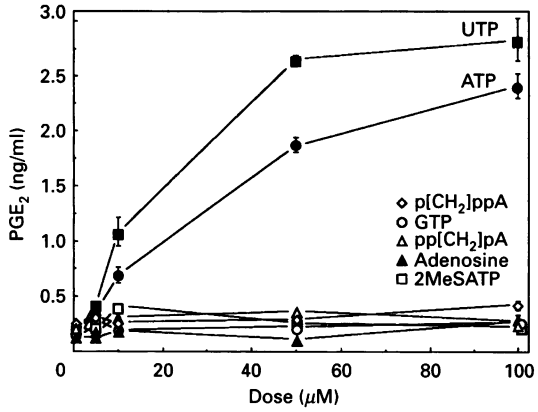

Figure 2 Dose-dependent increase in PGE_2 production by ATP and UTP in TEA3A1 cells

Confluent TEA3A1 cells were incubated for 15 min at 37 °C with increasing doses of indicated compounds. At the end of the experiment, media were collected and the level of PGE_2 produced by the cells was determined by RIA. Each point represents the mean ± S.D. ($n = 3$).

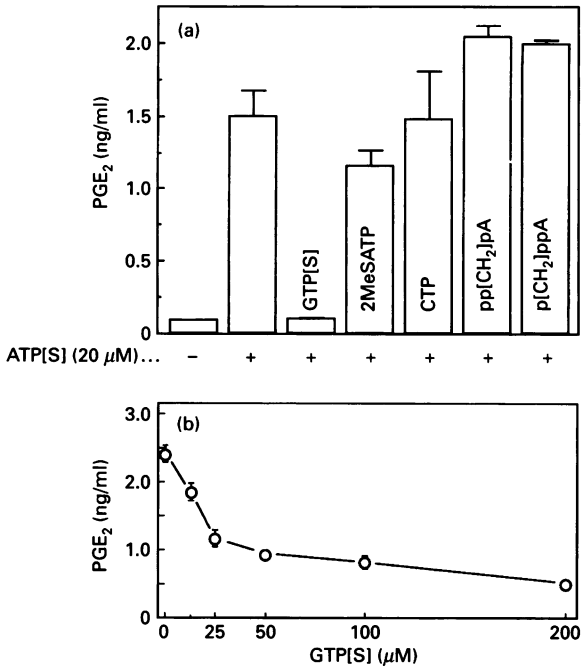

Figure 3 Inhibition of ATP[S]-mediated stimulation of PGE_2 production by GTP[S]

(**a**) The effect of various nucleotides on ATP[S]-mediated stimulation of PGE_2 production was examined. Cells were treated with 20 μM ATP[S] in the presence of 200 μM of the indicated nucleotide for 15 min. Each bar represents the mean ± S.D. ($n = 3$). (**b**) PGE_2 production stimulated by 20 μM ATP[S] was inhibited in a dose-dependent manner by GTP[S]. Cells were treated with 20 μM ATP[S] in the presence of the indicated amount of GTP[S] for 15 min. Each point represent the mean ± S.D. ($n = 3$).

subclasses of P2 receptors have been defined, based on the difference in the biological responses to various analogues of ATP and to other nucleotides, dose–response experiments were carried out by incubating TEA3A1 cells for 15 min with various nucleotide analogues. As shown in Figure 2, among the compounds tested, only ATP and UTP were able to stimulate PGE_2

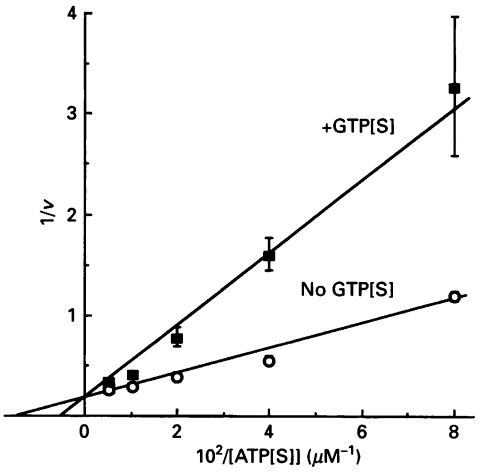

Figure 4 Competitive inhibition of ATP[S]-mediated stimulation of PGE_2 production by GTP[S]

PGE_2 production was measured in cells incubated with various doses of ATP[S] (3.125–200 μM) in the absence or presence of 50 μM GTP[S]. The reciprocal of the amount of PGE_2 produced (ng/ml in 15 min) at the point ($1/v$) was plotted against the reciprocal of the concentration of ATP[S] used. Lines were drawn by linear regression analyses ($r = 0.999$ for experiments in the presence of GTP[S]; $r = 0.989$ for experiments in the absence of GTP[S]). Each point represents the mean ± S.D. ($n = 3$).

production by TEA3A1 cells. Moreover, at each dose tested (5, 10, 50 and 100 μM), UTP was a more effective stimulator than ATP. Up to 50% more PGE_2 was produced by the cells stimulated by UTP than by ATP. ATP[S], a non-hydrolysable ATP analogue, was also able to stimulate PGE_2 production at a level equal to that observed with UTP (results not shown). None of the other nucleotide analogues was able to stimulate the production of PGE_2 by TEA3A1 cells within the doses tested (up to 100 μM). These results indicate that PGE_2 production by TEA3A1 cells is regulated by a P2 family of nucleotide receptors with agonist potencies of ATP[S] = UTP ⩾ ATP. Since ATP[S] is a non-hydrolysable analogue of ATP with a potent effect on PGE_2 production, all the following experiments were carried out using ATP[S] unless indicated otherwise.

GTP[S] inhibits ATP[S]-mediated stimulation of PGE_2 production

In order to determine further whether the nucleotides and analogues tested had any effect on the modulation of PGE_2 production, we examined whether these compounds could inhibit PGE_2 production stimulated by ATP[S]. As shown in Figure 3(a), when 200 μM GTP[S], 2MeSATP, CTP, pp[CH_2]pA or p[CH_2]ppA was added to cells treated with 20 μM ATP[S], it was found that GTP[S] could completely inhibit PGE_2 production stimulated by ATP[S]. A small inhibition of PGE_2 production (20%) was also observed with 2MeSATP. GTP was also able to inhibit ATP[S]-mediated stimulation of PGE_2 production with the same potency as GTP[S] (results not shown). Since GTP[S] is a non-hydrolysable analogue of GTP, GTP[S] was routinely used in our experiments. ATP[S]-mediated stimulation of PGE_2 production was inhibited by GTP[S] in a dose-dependent manner, with an ED_{50} of about 25 μM (Figure 3b). The kinetics of this GTP[S]-mediated inhibition of PGE_2 production was competitive, indicating that GTP[S] is competing at the ATP[S]-binding site of the receptor (Figure 4).

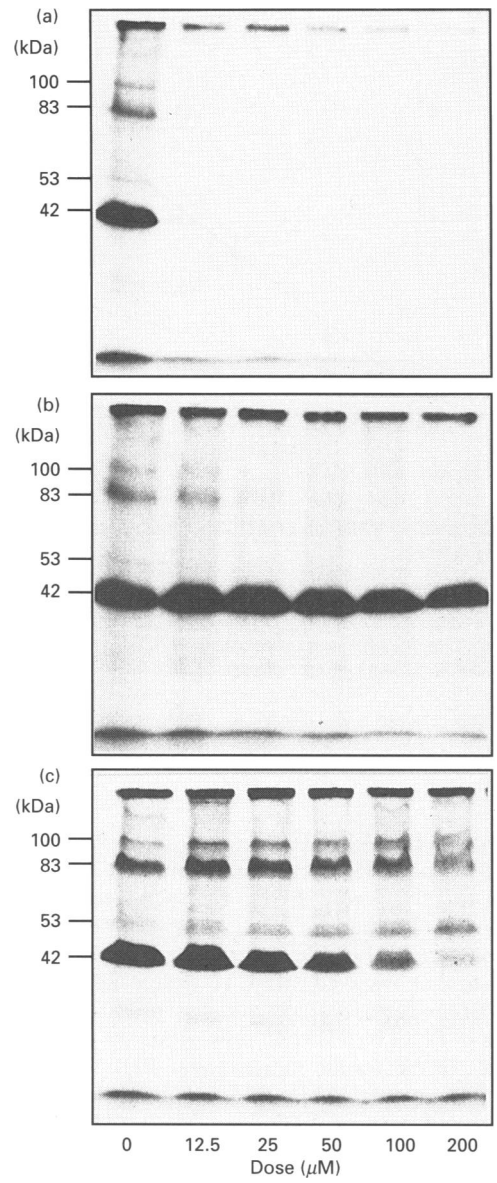

Figure 5 Fluorography of cell-surface ATP-binding sites cross-linked with ATP[^{35}S] in TEA3A1 cells in the presence of ATP[S], GTP[S] or 2MeSATP

Cell-surface ATP-binding sites on TEA3A1 cells are shown by fluorography after ATP[^{35}S] cross-linking to the cells in the absence (0) or presence of 12.5, 25, 50, 100 or 200 μM each of ATP[S] (**a**), GTP[S] (**b**) or 2MeSATP (**c**). Experiments were carried out as described in the Materials and methods section.

Cross-linking of ATP[^{35}S]

We have recently established methods to cross-link chemically ATP[^{35}S] bound to TEA3A1 cells in culture, using the homobifunctional cross-linker DMP [31]. Results from the above studies suggested that the binding of ATP[^{35}S] should be competed not only by excess of unlabelled ATP[S], ATP and UTP, but also by GTP[S]. In order to test this, ATP[^{35}S] cross-linking experiments were carried out in the presence or absence of nucleotides and analogues tested in the previous section. Among the nucleotides tested, GTP[S] and 2MeSATP showed selective inhibition of ATP[^{35}S] cross-linking. As shown in Figure 5, four protein bands, of molecular masses 42 kDa, 53 kDa, 83 kDa and 100 kDa, cross-linked with ATP[^{35}S] were readily identified by fluorography. Excess of unlabelled ATP as well as ATP[S] were able to compete with ATP[^{35}S] cross-linking to all these cross-linked protein species. Figure 5(a) shows results from adding excess of unlabelled ATP[S] at the indicated concentrations.

When ATP[^{35}S] cross-linking was carried out in the presence of ATP analogues and other nucleotides, we found that GTP[S] and 2MeSATP competed with ATP[^{35}S] cross-linking in a selective manner. As shown in Figure 5(b), treatment of cells with GTP[S] at the indicated concentrations blocked ATP[^{35}S] cross-linking to the 53 kDa, 83 kDa and 100 kDa proteins. Cross-linking of ATP[^{35}S] to the 42 kDa protein was not affected in the presence of excess of unlabelled GTP[S]. GTP also had an identical effect (results not shown). As shown in Figure 5(c), treatment of cells with excess of unlabelled 2MeSATP blocked ATP[^{35}S] cross-linking to the 42 kDa band in a dose-dependent manner, whereas ATP[^{35}S] cross-linking to the 83 kDa and 100 kDa proteins was not affected except when 200 μM 2MeSATP was added. In the presence of 200 μM 2MeSATP, cross-linking of ATP[^{35}S] to the 53 kDa band was slightly increased. UTP was able to compete partially with ATP[^{35}S] cross-linking. However, the competition was not selective, and the cross-linking of ATP[^{35}S] was decreased in all of the four protein bands (results not shown). Other nucleotides (CTP and p[CH_2]ppA at 200 μM) had little or no effect on ATP[^{35}S] cross-linking to the four proteins (CTP decreased the ATP[^{35}S] cross-linking to the 100 kDa protein, without affecting cross-linking to the other three bands, and p[CH_2]ppA had no effect on ATP[^{35}S] cross-linking to all four bands).

Since GTP and GTP[S] inhibited ATP[S]-mediated stimulation of PGE_2 production and cross-linking of ATP[^{35}S] to the 53 kDa, 83 kDa and 100 kDa proteins, these results would suggest that the ATP receptor responsible for the activation of PLA_2 enzymic activity in TEA3A1 cells is one of these three proteins. To examine this possibility further, we used a variant cell line, called TEAvar1, which we have isolated from TEA3A1 cells. TEAvar1 cells do not produce PGE_2 in response to ATP (Table 1). In these cells, PLA_2 and cyclo-oxygenase enzymic activities are unaffected, since PGE_2 production is stimulated when cells are treated with the Ca^{2+} ionophore A23187 (Table 1). Cross-linking experiments with TEAvar1 cells shows three bands of ATP[^{35}S]-cross-linked proteins, with apparent molecular masses of 100 kDa, 53 kDa and 42 kDa respectively (Figure 6). The presence of the 83 kDa ATP[^{35}S]-cross-linked protein found in the TEA3A1 cells was not detected in TEAvar1 cells. Moreover, the intensity of ATP[^{35}S] cross-linking to the 100 kDa band in TEAvar1 cells was decreased somewhat compared with the parent TEA3A1 cells. Results from these experiments would suggest that the 83 kDa protein is a possible candidate for the ATP receptor

Table 1 Stimulation of PGE_2 production by Ca^{2+} ionophore in TEAvar1 cells

Confluent TEAvar1 cells were incubated for 15 min in the presence or absence of either ATP[S] (20 μM) or A23187 (1 μg/ml). Media were harvested and the level of PGE_2 production was determined by RIA as described in the Materials and methods section. Numbers represent the mean $\pm$ S.D. ($n = 3$).

Treatment	PGE_2 (ng/ml)
None	0.09 ± 0.01
ATP[S] (20 μM)	0.12 ± 0.01
A23187 (1 μg/ml)	2.83 ± 0.07

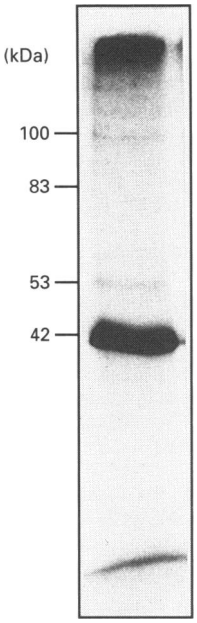

Figure 6 Fluorography of cell-surface ATP-binding sites cross-linked with ATP[^{35}S] in TEAvar1 cells

Cell-surface ATP-binding sites on TEAvar1 cells are shown by fluorography after ATP[^{35}S] cross-linking to the cells. ATP[^{35}S] cross-linking was carried out as described in the Materials and methods section. Only the 42 kDa band cross-linked with ATP[^{35}S] is present in these cells.

responsible for the stimulation of PGE_2 production in TEA3A1 cells.

DISCUSSION

ATP-mediated stimulation of AA metabolism has been reported in many experimental systems [35–39]. ATP-receptor-mediated signalling leading to the activation of PLA_2 enzymic activity has been well studied with HL60 cells, which express the P2u receptor [19]. The P2u receptor is coupled to a pertussis-toxin-sensitive G-protein and mediates its signal via generation of $InsP_3$ [40,41]. The recently cloned P2u receptor cDNA codes for a 42 kDa protein and is expressed as a 53 kDa glycosylated protein in cells transfected with an expression vector containing P2u cDNA [26,27]. The relative agonist potency for the P2u receptor is ATP = UTP > ATP[S]. It has been shown that GTP when added at high concentrations can also act as an agonist for the P2u receptor [19].

Using TEA3A1 rat thymic epithelial cells, we showed that stimulation of PGE_2 production took place with relative agonist potency of ATP[S] $\geqslant$ UTP > ATP, with ED_{50} of 10 μM with ATP[S]. Interestingly, GTP and GTP[S] act as potent antagonists for the ATP[S] effect on PGE_2 production in TEA3A1 cells, with IC_{50} of about 25 mM. Our results indicate that GTP[S] inhibited ATP[S]-mediated PGE_2 production in a competitive manner, suggesting that GTP[S] is competing with ATP[S] at the receptor-binding site. GTP[S] was also able to block UTP-mediated stimulation of PGE_2 production with equal potency (results not shown).

From the ATP[^{35}S] cross-linking studies carried out in the presence of GTP[S] and 2MeSATP using TEA3A1 and TEAvar1 cells, we were able to establish a correlation between the presence of an 83 kDa protein cross-linked to ATP[^{35}S] and the stimulation of PGE_2 production by ATP. Although UTP is a potent agonist for PGE_2 production, UTP competed poorly with ATP[^{35}S] cross-linking and was only able to decrease partially the overall cross-linking of ATP[^{35}S] when added at a high concentration. In this respect, the results from ATP[^{35}S] cross-linking competition studies did not strictly reflect the agonist potencies of the nucleotide analogues. Similar observations were made recently with cloned cDNA for the P2u receptor expressed in K-562 cells, where UTP failed to compete with [α-^{32}P]3′-*O*-(4-benzoyl-benzoyl)ATP binding [27].

Our results indicated that ATP-mediated stimulation of AA metabolism in TEA3A1 cells was not mediated by a typical P2 receptor. The ATP receptor responsible for the activation of PLA_2 enzymic activity in TEA3A1 cells is similar to P2u with respect to agonist potency. However, the key feature separating this ATP receptor from the previously described P2u receptor is the fact that GTP and GTP[S] are effective specific antagonists in our studies, whereas GTP is known to activate the P2u receptor when added at high concentrations [19]. In TEA3A1 cells, GTP and GTP[S] can specifically block ATP-mediated activation of PLA_2 and PGE_2 production and block ATP[^{35}S] cross-linking to the 53 kDa, 83 kDa and 100 kDa proteins. In a variant cell line of TEA3A1, TEAvar1 cells, ATP[S] treatment of the cells does not lead to the stimulation of PGE_2 production. The lack of ATP[S]-mediated stimulation of PGE_2 production by these cells is not due to the lack of PLA_2 or cyclo-oxygenase enzymic activities, since PGE_2 production can be stimulated by the treatment of cells with the Ca^{2+} ionophore A23187. In TEAvar1 cells, ATP[^{35}S] cross-linked to 42 kDa, 53 kDa and 100 kDa proteins. The 83 kDa protein that cross-linked with ATP[^{35}S] found in TEA3A1 cells was not detected in TEAvar1 cells. Thus, assuming that the ATP receptor responsible for the activation of PLA_2 enzymic activity in TEA3A1 is cross-linked with ATP[^{35}S], it appears that the 83 kDa protein is a possible candidate for the ATP receptor responsible for the activation of PLA_2 enzymic activity in TEA3A1 cells. However, it is not possible to draw any definitive conclusion from our present studies.

In HL60 cells, where the presence of P2u has been established, it has been shown that GTP at high concentration can stimulate AA metabolism [19]. When we carried out cross-linking of ATP[^{35}S] to surface ATP-binding proteins of dibutyryl-cyclic-AMP-treated differentiated HL60 cells, we found the presence of 47, 56 and 62 kDa cross-linked bands which were not readily detectable in undifferentiated HL60 cells. ATP[^{35}S]-cross-linked 83 kDa protein was absent from HL60 cells (P. Liu and J. Hayashi, unpublished work). Among these cross-linked bands, cross-linking of ATP[^{35}S] to 47 kDa and 56 kDa proteins was partially competed by UTP. Since HL60 is a human cell line, we have also examined rat adipocytes in primary cultures where the activation of AA mobilization takes place upon binding of ATP to the P2u receptor (G. Serrero, personal communication) Again, GTP or GTP[S] had no inhibitory effect on ATP-mediated activation of AA metabolism in these cells. On the contrary, GTP[S] at higher dose stimulated AA metabolism in these cells. ATP[^{35}S] cross-linking experiments with rat adipocytes revealed the presence of 44, 54 and 70 kDa bands. The presence of an 83 kDa cross-linked protein was not detected in these cells (P. Liu, M. Wen and J. Hayashi, unpublished work).

Our results, together with these supporting data from HL60 cells and rat adipocytes, would indicate that the ATP receptor responsible for the activation of PLA_2 enzymic activity in TEA3A1 cells is different from the previously described P2u receptor or from any of the other previously characterized purinergic receptors. The role of ATP in thymic epithelial cells has not been studied to date. We believe that ATP plays an essential role in the regulation of PGE_2 production by activating

PLA_2 enzymic activity in thymic epithelial cells. The injection of ATP and ATP[S] into thymic lobes results in an immediate increase in the production of PGE_2 (D. Lalor, P. S. Liu and J. Hayashi, unpublished work), indicating the physiological role that ATP plays in the thymus.

A likely source of ATP in thymus is neurons. Modulation of lymphoid cell development and function by the nervous system has been postulated for some time. In recent years, the existence of noradrenergic sympathetic nerves and cholinergic parasympathetic nerves has been identified in the thymus [42–44]. Innervation of primary lymphoid organs is an important developmental step that must take place before the onset of lymphopoiesis [45]. ATP has been reported to be packaged with cholinergic and catecholaminergic neurotransmitters in storage vesicles and co-released during the process of nerve stimulation [46]. These observations suggest that the levels of PGE_2 in the thymus may be under neuronal control.

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