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Extracellular ATP causes lysis of mouse thymocytes and activates a plasma membrane ion channel

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Extracellular ATP (ATP_o) caused a concentration-dependent lysis of mouse thymocytes. Lysis, as judged by release of the cytosolic enzyme lactate dehydrogenase, was preceded by depolarization of the plasma membrane and by Ca^{2+} influx. Both Na^+ uptake (which sustained plasma membrane depolarization) and Ca^{2+} influx showed (1) the same dependence on the ATP_o concentration; (2) the same nucleotide specificity; and (3) the same Hill coefficient. However, whereas the rise in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) was fully inhibited by the known Ca^{2+} blocker verapamil, plasma membrane depolarization was enhanced under these conditions. Plasma membrane depolarization was greater and was shifted to lower ATP_o concentrations in the absence of extracellular Ca^{2+} (Ca^{2+}_o), whereas the rise in $[Ca^{2+}]_i$ was greater in Na^+-free media. Plasma membrane depolarization also occurred in Na^+-free choline- or methylglucamine-containing media, and was potentiated by chelation of free divalent ions with EDTA, supporting previous reports pointing to ATP^{4-} as the active species. Among a number of purine and pyrimidine nucleotides, only adenosine 5′-[γ-thio]triphosphate and ADP were partially effective. Furthermore, ethidium bromide (M_r 380), Lucifer Yellow (M_r 463) and Eosin Yellowish (M_r 692) did not permeate through the ATP_o-activated channel. These findings suggest that lytic effects of ATP_o in mouse thymocytes depend on the activation of a membrane channel with low selectivity for cations and an M_r cut-off of 200.

INTRODUCTION

Several effects of extracellular ATP (ATP_o) on cellular physiology have been described [1–4]. However, only recently has attention been paid to the long-standing observation that ATP_o is also cytolytic [5–9]. The lytic properties of ATP_o are particularly prominent in lymphocytes and lymphoid cells, in which even short exposures cause irreversible damage to the plasma membrane and release of cytosolic enzymes. ATP_o-dependent cytolysis is accompanied in some cell lines by DNA fragmentation [9], a picture reminiscent of the nuclear alteration of target cells lysed by cytotoxic T lymphocytes. Therefore a role for ATP, as a soluble factor of cell-mediated cytotoxicity has recently been proposed [6,7,10]. The intracellular mechanisms whereby ATP_o, as well as other cytotoxic factors, triggers DNA fragmentation and cell lysis are unknown, although a role for increases in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) has been proposed [11].

In order to identify the intracellular messengers that mediate ATP_o-dependent DNA fragmentation and cell lysis, we investigated the early alterations in intracellular ion homeostasis and plasma membrane permeability caused by this nucleotide. We were unable to detect in ATP_o-treated lymphocytes and other lymphoid cells the early and non-specific increases in plasma membrane permeability described in mast cells, fibroblasts and macrophages [12–14]. Uptake of extracellular markers such as ethidium bromide, Lucifer Yellow and Eosin Yellowish occurred only after 30–60 min, coincident with lactate dehydrogenase release, thus indicating non-specific membrane damage.

However, ATP_o activated membrane channel(s) which were permeable to Na^+, Ca^{2+}, choline (M_r 100) and methylglucamine (M_r 190), thus causing a depolarization of plasma membrane potential and a rise in $[Ca^{2+}]_i$. Although some of these properties are similar to those of the channel described by Benham & Tsien in smooth muscle cells [15], the ligand-selectivity differed, as the effective species was ATP^{4-} and not $MgATP^{2-}$. Furthermore, the channel(s) of lymphocytes also differed from those described by Buisman *et al.* in macrophages [16] and by Tatham & Lindau in mast cells [17], having a much lower M_r cut-off. Finally, in thymocytes, in contrast with reports on other cell types [18–21], ATP_o was unable to generate $InsP_3$ and mobilize Ca^{2+} from intracellular stores [22], thus suggesting a direct coupling of the ATP_o receptor to the channel(s). However, a rise in $[Ca^{2+}]_i$ was not required for lysis, thus indicating that ATP_o activates a Ca^{2+}- independent lytic pathway in mouse thymocytes.

EXPERIMENTAL

Cells

Thymocytes were prepared from 3-week-old BALB/c mice as previously described [23], and kept in a medium containing (in mmol/litre): 125 NaCl, 5 KCl, 1 $MgSO_4$, 1 Na_2HPO_4, 5.5 glucose, 5 $NaHCO_3$, 1 $CaCl_2$ and 20 Hepes (pH 7.4, 37 °C). This saline medium was used, unless otherwise indicated, for all experiments, and is hereafter referred to as standard saline. In some experiments NaCl was replaced by an iso-osmotic concentration of choline chloride, methylglucamine or sucrose. In these sodium-free buffers KCl was omitted, Na_2HPO_4 was replaced with K_2HPO_4, and $NaHCO_3$ with $KHCO_3$, and the pH was adjusted

Abbreviations used: ATP_o, extracellular ATP; Ca^{2+}_i, intracellular free Ca^{2+}; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; Ca^{2+}_o, extracellular free Ca^{2+}; $[Ca^{2+}]_o$, extracellular free Ca^{2+} concentration; fura-2/AM, fura-2 acetoxymethyl ester; VOCs, voltage-operated Ca^{2+} channels; ROCs, receptor-operated Ca^{2+} channels; ATP[S], adenosine 5′-[γ-thio]triphosphate; pp[CH_2]pA, adenosine 5′-[αβ-methylene]triphosphate; p[NH]ppA, adenosine 5′-[βγ-imido]triphosphate; Ap_4A, diadenosine tetraphosphate.

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to 7.4 with Tris/HCl. The J774 mouse macrophage-like cell line was grown as previously described [14].

Measurement of $[Ca^{2+}]_i$

Loading with fura-2/AM and measurement of $[Ca^{2+}]_i$ were performed essentially as previously described [24]. For measurement of $[Ca^{2+}]_i$, thymocytes were suspended in a thermostatted and magnetically stirred fluorimeter (Perkin–Elmer LS5) cuvette at a concentration of 2×10^6 cells/ml.

Measurement of plasma membrane potential

The measurement of membrane potential using bis-oxonol (100 nM), at the wavelength pair 540–580 nm, was performed as previously described [25]. The fluorescence response was calibrated with gramicidin D or, in Na^+-free medium, by sequential additions of 15 mM-KCl.

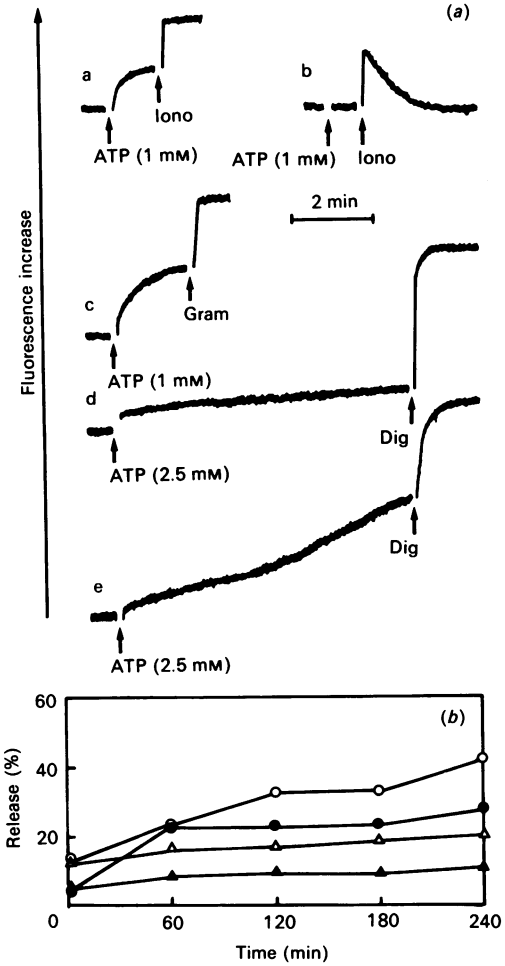

Fig. 1. ATP_o causes Ca^{2+} influx, plasma membrane depolarization and delayed lysis

(*a*) Thymocytes were suspended at a concentration of 2×10^6 cells/ml in standard saline (traces a, c and d) or in Ca^{2+}-free saline containing 100 μM-EGTA (trace b). In traces a and b, thymocytes were also loaded with 2 μM-fura-2/AM, whereas in traces c and d they were incubated in the presence of 100 nM-bis-oxonol and 20 μM-ethidium bromide respectively. Trace e reports ethidium bromide (20 μM) uptake from J774 mouse macrophages suspended in standard saline at a cell concentration of 10^6/ml. Ionomycin (Iono), gramicidin D (Gram) and digitonin (Dig) concentrations were 100 nM, 500 nM and 20 μM respectively. (*b*) Time course of lactate dehydrogenase release from 10^6 control (▲, △) or ATP_o (1 mM)-treated (●, ○) thymocytes incubated in the presence (▲, ●) or the absence (△, ○) of 1 mM-Ca^{2+}.

Enzyme release

Lactate dehydrogenase activity was measured according to standard methods [26].

Calculation of $[ATP^{4-}]$

The ATP^{4-} concentration, as a function of total ATP_o, Ca^{2+} and Mg^{2+} concentrations and pH, was determined according to Fabiato [27].

Chemicals

ATP, ADP, GTP, ITP, UTP, CTP, adenosine 5′-[γ-thio]triphosphate (ATP[S]), adenosine 5′-[α,β-methylene] triphosphate (pp[CH_2]pA) and adenosine 5′-[βγ-imido]triphosphate (p[NH]ppA) were purchased from Boehringer Mannheim; fura-2/AM and bis-oxonol were from Molecular Probes. Pertussis toxin was a gift from Dr. R. Rappuoli, Sclavo Research Laboratories, Siena, Italy.

RESULTS

Fig. 1 shows the kinetics of the rise in $[Ca^{2+}]_i$ (Fig. 1*a*, traces a and b), plasma membrane depolarization (trace c), ethidium bromide uptake (trace d) and lactate dehydrogenase release (Fig. 1*b*) caused by ATP_o in mouse thymocytes. The $[Ca^{2+}]_i$ rise and depolarization were both triggered without an appreciable lag and reached a maximum within 30–60 s. On the contrary, uptake of ethidium bromide was negligible for at least 10 min after the addition of ATP_o. Trace e shows for comparison that, in J774 mouse macrophages, a cell type known to possess ATP_o-gated channels permeable to external M_r markers up to 900, exposure to ATP_o for a similar time caused a 60% uptake of ethidium bromide. Fig. 1(*b*) shows that, although depolarization and a $[Ca^{2+}]_i$ rise were the only detectable early changes in ATP_o-challenged thymocytes, after 1 h lactate dehydrogenase had also started to leak out, thus indicating lysis. Increased ethidium

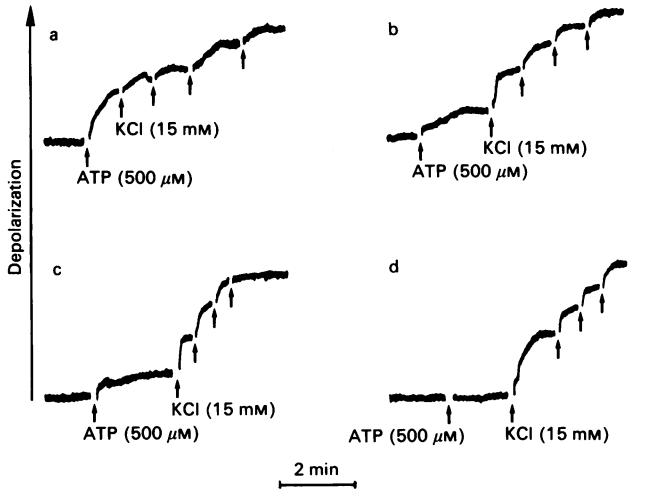

Fig. 2. ATP_o causes depolarization in the presence and absence of extracellular Na^+

Thymocytes were suspended at a concentration of 2×10^6 cells/ml in one of the following incubation media containing 100 nM-bis-oxonol: standard saline (trace a); Na^+-free 125 mM-choline-supplemented saline (trace b); Na^+-free 125 mM-methylglucamine-supplemented saline (trace c); Na^+-free 250 mM-sucrose-supplemented saline (trace d). Changes in plasma membrane potential were calibrated by sequential additions of 15 mM-KCl (arrows).

bromide uptake was simultaneous with lactate dehydrogenase release at this late stage (results not shown). Fig. 1(*a*), trace b indicates that ATP_o was unable to mobilize Ca^{2+} from intracellular stores, although, as demonstrated by the $[Ca^{2+}]_i$ increase caused by ionomycin, thymocytes possess releasable intracellular Ca^{2+} stores. Nonetheless, as shown in Fig. 1(*b*), ATP_o also caused lysis in the absence of $Ca^{2+}{}_o$, and therefore under conditions preventing a rise in $[Ca^{2+}]_i$. Although mouse thymocytes contain Ca^{2+}-activated K^+ channels which are responsive to the rise in $[Ca^{2+}]_i$ elicited by ionomycin (results not shown), no hyperpolarization was elicited by ATP_o, presumably because of the simultaneous stimulation of a depolarizing Na^+ influx.

ATP_o triggered a depolarization of plasma membrane potential (Fig. 2, trace a) that was also observed, albeit decreased by about 60%, in media in which Na^+ was replaced by iso-osmotic choline or methylglucamine (traces b and c respectively). On the contrary, complete substitution of Na^+ with sucrose (trace d) prevented the ATP_o-induced depolarization, but not that induced by KCl.

The ATP_o thresholds for the $[Ca^{2+}]_i$ increase and depolarization were 10 μM and 100 μM respectively, whereas the concentration giving a half-maximal effect (ED_{50}) was 200 μM for both (Figs. 3*a* and 3*b*). There was an apparent optimal ATP_o concentration (about 650–800 μM) for the $[Ca^{2+}]_i$ rise, above which the increase in $[Ca^{2+}]_i$ declined. On the contrary, no such decline was observed for plasma membrane depolarization at high ATP_o concentrations. Increasing the $[ATP]_o$ from 650 μM to 1 mM decreased $[Ca^{2+}]_o$ from 0.86 to 0.63 mM, as calculated according to the computer program elaborated by Fabiato [27]; it is therefore likely that the decrease in $[Ca^{2+}]_i$ at ATP_o concentrations above 800 μM was caused by the decrease in $[Ca^{2+}]_o$. The ATP_o dose-dependence curves for both the $[Ca^{2+}]_i$ increase and plasma membrane depolarization indicate a co-operative activation of the ATP_o-dependent channel. In Fig. 3, Hill plots for the rise in $[Ca^{2+}]_i$ (Fig. 3*c*) and depolarization (Fig. 3*d*) are also shown. Hill coefficients calculated from these plots were 2.00 and 2.23 for $[Ca^{2+}]_i$ rise and depolarization respectively, suggesting that, in agreement with a recent report on mast cells [17], at least two ATP molecules must ligate the surface receptor(s) to cause Ca^{2+} entry and depolarization.

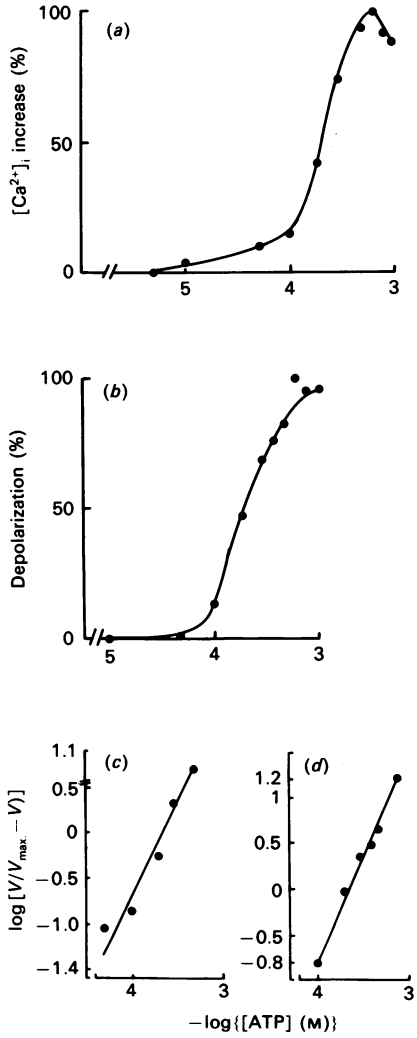

Fig. 3. ATP_o dose–response for $[Ca^{2+}]_i$ increases and plasma membrane depolarization

See the legends to Figs. 1 and 2 for experimental conditions. Increases are expressed as percentage of maximal increase caused by 650 μM- and 1 mM-ATP_o for the rise in $[Ca^{2+}]_i$ and depolarization respectively. (*c*) and (*d*) represent Hill plots of (*a*) and (*b*) respectively. V is the rate of $[Ca^{2+}]_i$ increase or depolarization observed at each ATP_o concentration and $V_{max.}$ is the rate at a maximal $[ATP]_o$.

$Ca^{2+}{}_o$ was not required for ATP_o-dependent depolarization, and was in fact inhibitory (compare traces a, b and c in Fig. 4). In the presence of 1 mM- (trace b) or 4 mM- (trace c) $[Ca^{2+}]_o$ respectively, depolarization was decreased by 70% or completely abolished compared with that observed in a Ca^{2+}-free EGTA-supplemented medium (trace a). Several reports suggest that the active ATP_o species that triggers Na^+ and Ca^{2+} influx is ATP^{4-} [6,12,14,18]; it is therefore not surprising that raising the $[Ca^{2+}]_o$ decreased ATP_o-dependent depolarization, since the concentration of the Ca–ATP^{2-} chelate increased at the expense of ATP^{4-}. However, in the presence of a $[Ca^{2+}]_o$ of 4 mM, 650 μM-ATP_o caused a maximal rise in $[Ca^{2+}]_i$ (results not shown), thus indicating activation of the ion channel, yet depolarization was prevented. Therefore it cannot be excluded that the decreased depolarization in the presence of a high $[Ca^{2+}]_o$ could be due to competition for entry between Na^+ and Ca^{2+}. If this were true, then the rise in $[Ca^{2+}]_i$ should also be affected by the Na^+ concentration in the medium.

To test this hypothesis, we investigated the ATP_o-induced rises in $[Ca^{2+}]_i$ in thymocytes suspended in standard saline (Fig. 5, trace a) or in Na^+-free methylglucamine- (trace b) or sucrose- (trace c) supplemented saline. The steady-state rise in $[Ca^{2+}]_i$ was enhanced 2-fold in the presence of methylglucamine, a cation less permeant than Na^+, and 3-fold in the presence of sucrose, a substituent fully impermeant across the ATP_o-gated channel. Incubation in methylglucamine or sucrose also caused a rise in the prestimulated $[Ca^{2+}]_i$, and acceleration of the kinetics of the

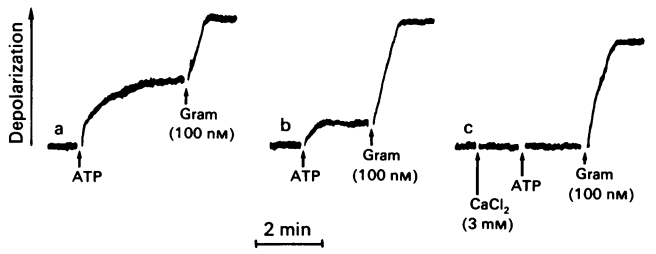

Fig. 4. Extracellular Ca^{2+} inhibits plasma membrane depolarization

Thymocytes (2×10^6 cells/ml) were incubated in the presence of 100 nM-bis-oxonol in Ca^{2+}-free saline containing 100 μM-EGTA (trace a), in standard saline (trace b) or in standard saline supplemented with 3 mM-$CaCl_2$ (trace c). $[ATP]_o$ was 650 μM. Gram, gramicidin D.

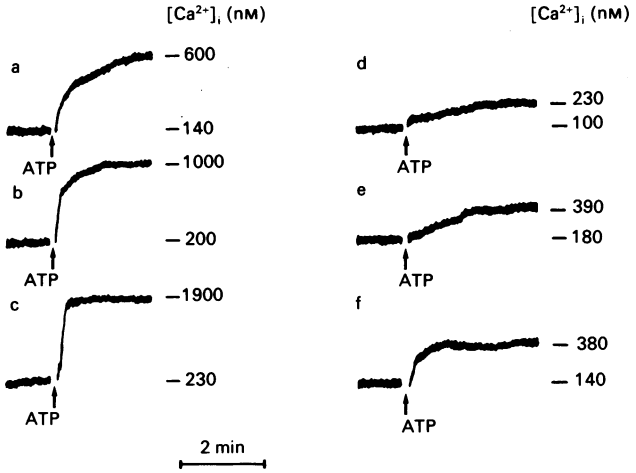

Fig. 5. Effects of Na^+ substitution on the ATP_o-triggered rise in $[Ca^{2+}]_i$

Thymocytes (2×10^6 cells/ml), loaded with 2 μM-fura-2/AM, were suspended in the following media: standard saline (traces a and d), Na^+-free saline supplemented with 125 mM-methylglucamine (traces b and e), or Na^+-free saline supplemented with 250 mM-sucrose (traces c and f). In traces d, e and f, thymocytes were also depolarized with 60 mM-KCl before the addition of ATP (650 μM).

$[Ca^{2+}]_i$ rise, probably as a consequence of a decreased activity of the Na^+/Ca^{2+} exchanger. In the absence of extracellular Na^+ the plasma membrane potential is hyperpolarized, and therefore the driving force for Ca^{2+} entry is greater. To rule out a major contribution of a hyperpolarized plasma membrane potential in sustaining a larger Ca^{2+} influx in Na^+-free media, we depolarized thymocytes suspended in Na^+, methylglucamine or sucrose with 60 mM-KCl before the addition of ATP_o. As shown in Fig. 5 (traces d, e and f) pre-depolarization decreased the ATP_o-induced rises in $[Ca^{2+}]_i$, as is expected for an electrogenic Ca^{2+} influx through a membrane channel. However, in these KCl-depolarized cells the rise in $[Ca^{2+}]_i$ was also higher in the absence of Na^+, suggesting an ancillary role of a hyperpolarized plasma membrane potential.

Fig. 6 reports the dependence of the ATP_o-triggered $[Ca^{2+}]_i$ rise on the Na^+ and sucrose concentrations, showing that a

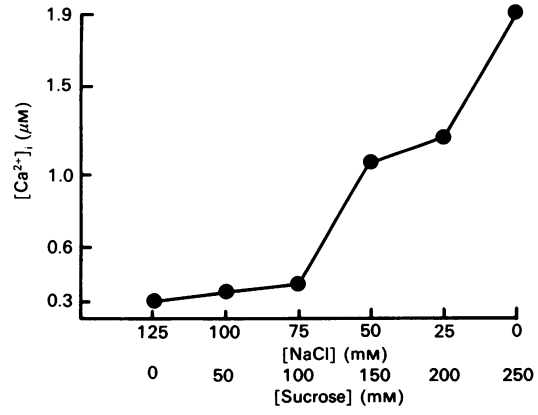

Fig. 6. Dependency of the rise in $[Ca^{2+}]_i$ on the extracellular Na^+ concentration

Experimental conditions were as in Fig. 5. Thymocytes (2×10^6/ml) were loaded with 2 μM-fura-2/AM and incubated in saline in which Na^+ was gradually replaced by iso-osmotic sucrose, as indicated.

Table 1. Effect of various nucleotides on plasma membrane depolarization and increase in $[Ca^{2+}]_i$

Plasma membrane potential and $[Ca^{2+}]_i$ changes were measured in standard saline as described in the legends to Figs. 1 and 2. The nucleotide concentration was 1 mM.

Nucleotide	Depolarization (% of maximal)	$[Ca^{2+}]_i$ increase (% of maximal)
ATP	100	100
Adenosine	0	0
AMP	0	0
ADP	34	10
Ap_4A	0	0
ATP[S]	70	70
p[NH]ppA	0	0
pp[CH_2]pA	0	0
GTP	0	0
ITP	0	0
UTP	0	0
CTP	0	0

potentiation of Ca^{2+} influx occurred at extracellular Na^+ concentrations below 75 mM.

Both Ca^{2+} influx and plasma membrane depolarization were activated only by ATP, ADP and ATP[S]; all other nucleotides tested, as well as adenosine, were ineffective (Table 1). The ligand selectivity is therefore the same as that of the ATP_o receptor that modulates plasma membrane permeability in mast cells and macrophages [12,14]. Occasionally we observed that millimolar concentrations of UTP and CTP caused a small increase in $[Ca^{2+}]_i$ (less than 10% of that caused by ATP). This pyrimidine-nucleotide-triggered rise in $[Ca^{2+}]_i$ occurred very unpredictably, and probably depended on ligation of an additional receptor for extracellular nucleotides, possibly similar to that described by Greenberg *et al.* in macrophages [18].

Some agents known to block Ca^{2+} fluxes through receptor-operated channels (ROCs) or voltage-operated channels (VOCs)

Table 2. Effect of verapamil on ATP_o-induced depolarization and increase in $[Ca^{2+}]_i$

Plasma membrane potential and $[Ca^{2+}]_i$ changes were analysed as described in the legends to Figs. 1 and 2. Additions were ATP (100 μM), verapamil (10 μM) or concanavalin A (20 μg/ml). Thymocytes were suspended in either Ca^{2+}-free EGTA-supplemented saline or standard saline (containing 1 mM-Ca^{2+}). Changes in plasma membrane potential and in $[Ca^{2+}]_i$ are expressed as a percentage of maximal depolarization and $[Ca^{2+}]_i$ rises induced by either 650 μM-ATP or 20 μg of concanavalin A/ml respectively. Figures in parentheses report actual $[Ca^{2+}]_i$ values (in nM) after the additions indicated.

Additions	Depolarization (% of max.)		$[Ca^{2+}]_i$ increase (% of max.)
	Ca^{2+} free + EGTA (100 μM)	1 mM-Ca^{2+}	1 mM-Ca^{2+}
None	0	0	0 (170)
ATP	15	26	15 (200)
ATP + verapamil	57	52	0 (170)
Concanavalin A	–	–	100 (230)
Concanavalin A + verapamil	–	–	100 (230)

were tested as possible inhibitors of the ATP_o-activated channel(s). Preincubation with pertussis toxin (100 ng/ml) for up to 2–4 h had no effect on Ca^{2+} influx triggered by ATP_o (results not shown). Likewise, no effect was observed with the specific blockers of VOCs, nitrendipine (200 nM) and Ni^{2+} (200 μM) (results not shown). In contrast, the other well-known blocker of VOCs, verapamil, had a peculiar effect on $[Ca^{2+}]_i$ and plasma membrane depolarization. On the one hand it abolished ATP_o-triggered Ca^{2+} influx, and on the other it increased by more than 3-fold plasma membrane depolarization (Table 2). Lack of inhibition by verapamil of the rise in $[Ca^{2+}]_i$ caused by concanavalin A suggests that the verapamil effect was due not to a non-specific inhibition of Ca^{2+} entry, but rather to an interaction with the ATP_o-gated channel(s).

DISCUSSION

There is surging interest in the biological actions of ATP_o and on the intracellular transduction system activated by this nucleotide [28]. Among the many cellular responses elicited by ATP_o, cytolysis is the most intriguing, especially in the light of the early DNA fragmentation that accompanies, and in some cell types precedes, ATP_o-mediated lysis [9]. The pattern of ATP_o-dependent cell lysis is reminiscent of that of the internal disintegration observed during apoptosis and cell death mediated by cytotoxic cells. In the light of this observation and of the peculiar resistance of cytotoxic T cells to ATP_o, a role for this nucleotide as a possible mediator of cytotoxic lysis has been proposed [6,7,9,10].

The intracellular mediators of the lytic effects of ATP_o and other cytotoxic factors (e.g. tumour necrosis factor, lymphotoxin, perforins) are unknown, although a role for increases in $[Ca^{2+}]_i$ has been proposed [11]. ATP_o has been reported to cause depolarization of the plasma membrane potential [6,29], release of Ca^{2+} from intracellular stores [18,19], influx of Ca^{2+} from the extracellular medium [18,22], generation of $InsP_3$ [21], and uptake of extracellular aqueous solutes of M_r up to 900 [12,14]. In mouse thymocytes, however, ATP_o only caused depolarization of plasma membrane potential and Ca^{2+} influx. Both Na^+ and Ca^{2+} fluxes showed a similar dependence on the ATP_o concentration, the same nucleotide specificity and the same Hill coefficient. In addition, depolarization was greater and was shifted to lower ATP_o concentrations in the absence of $[Ca^{2+}]_o$. On the other hand, the increase in $[Ca^{2+}]_i$ was greater in the absence of Na^+, thus suggesting competition between Na^+ and Ca^{2+}. ATP, and to a lesser extent ATP[S] and ADP, was the only active nucleotide, and a pertussis toxin-sensitive GTP-binding protein appeared not to be involved. These properties are similar to those reported by Benham & Tsien in smooth muscle cells [15]; nonetheless, in contrast with the channel described by these latter authors, who found that $MgATP^{2-}$ and ATP^{4-} were equally effective, under our conditions the only active species was ATP^{4-}. However, the ATP_o-gated channel(s) of thymocytes also differs from that described by Tatham & Lindau in mast cells [17], as it does not admit molecules of M_r 324 and above.

A further intriguing feature was the modulation of cation permeability by verapamil. In fact, whereas other known blockers of voltage-gated Ca^{2+} channels, such as Ni^{2+} and nitrendipine, were ineffective, verapamil on the one hand fully blocked the ATP_o-dependent $[Ca^{2+}]_i$ rise and on the other potentiated Na^+ influx. This peculiar effect of verapamil might suggest either that Ca^{2+} and Na^+ fluxes occurred through two different ATP_o-activated channels with different sensitivities to verapamil, or that a shift in the selectivity of the channel from Ca^{2+} to Na^+ occurred in the presence of this Ca^{2+} antagonist.

In thymocytes, ATP_o caused neither generation of $InsP_3$ nor release of Ca^{2+} from internal stores; therefore the opening of the channel described in this report does not depend on the generation of known second messengers which are putatively involved in Ca^{2+} gating across the plasma membrane, such as $InsP_3$, $InsP_4$ or Ca^{2+} itself.

The disequilibrium in intracellular monovalent ion homeostasis appeared to be a main determinant of the lytic effects of ATP_o, as lysis was significantly lowered in Na^+-free sucrose-containing medium (P. Pizzo, unpublished work). On the contrary, and in contrast with previous results in hepatocytes [30], a rise in $[Ca^{2+}]_i$ was not needed for lysis of thymocytes incubated in standard saline solution.

The ATP_o receptor in thymocytes shares intriguing similarities with the receptor for excitatory amino acids in central neurons. This latter receptor also gates a channel that causes Ca^{2+} influx in the absence of Ca^{2+} mobilization from stores, triggers depolarization of plasma membrane potential, has low selectivity for Ca^{2+} over Na^+ and is putatively involved in cell death (excitotoxicity) [31]. It could be postulated that, as is the case in the central nervous system whereby excitatory amino acids serve both the function of neuromediators and, when they are released in large amounts, that of effectors of cell death, thus in the same way in the immune system ATP_o may be a mediator of cell-to-cell communication and, under some circumstances, an effector of cell death.

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