ELSEVIER

Biochimica et Biophysica Acta 1280 (1996) 217–222

BBA Biochimica et Biophysica Acta

Characterization of P_{2Z} purinergic receptors on phagocytic cells of the thymic reticulum in culture

Robson Coutinho-Silva [a], Luiz Anastácio Alves [a,b], Antônio Carlos Campos de Carvalho [a], Wilson Savino [b], Pedro Muanis Persechini [a,*]

[a] *Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Bloco G do CCS - Ilha do Fundão, 21941-900 Rio de Janeiro, Brazil*

[b] *Laboratório de Pesquisas Sobre o Timo, Instituto Oswaldo Cruz -FIOCRUZ, Rio de Janeiro, Brazil*

Received 24 October 1995; accepted 30 November 1995

Abstract

The thymic microenvironment is under intrinsic and extrinsic control circuits by several elements including hormones, neuropeptides, lymphokines, innervation and cell contact. P_2 purinergic receptors have been described in a number of cells including macrophages, thymocytes, and other cells of the immune-inflammatory system. Here, we use the whole-cell patch-clamp technique and dye permeabilization assays to investigate the presence of ionic channels and purinergic receptors in one microenvironmental thymic component, namely the phagocytic cell of the thymic reticulum. At holding potentials ranging from −30 to −60 mV, applications of extracellular ATP in the vicinity of the cell membrane induce a transient and fast-activating inward current followed in most cells by an outward current. The whole event lasts 5–20 s. The inward current has a reversal potential close to 0 mV and the outward current can be ascribed to a Ca^{2+}-dependent K^+ conductance. Both currents are inhibited by Mg^{2+}, suggesting that the phenomenon is mediated by ATP^{4-}. ATP-γ-S can also induce both inward and outward currents. Exposure of phagocytic cells of the thymic reticulum to 5 mM ATP for 10 min induced permeabilization to lucifer yellow but not to the larger dyes trypan blue and rhodamine-dextran, suggesting a molecular weight cut-off smaller than 900. These observations lead us to conclude that phagocytic cells of the thymic reticulum express P_{2Z} purinergic receptors that can mobilize Ca^{2+}, induce the opening of ionic channels and permeabilize the cell membrane.

Keywords: ATP; Purinergic receptor; Phagocytic cell; Thymic reticulum; Dendritic cell; Patch clamp

1. Introduction

A non-epithelial thymic microenvironmental cell, called phagocytic cell of the thymic reticulum (P-TR) has been previously described [1]. These cells share some characteristics with macrophages and interdigitating dendritic cells, such as: interleukin-1 and interleukin-2 receptors, CR3 complement receptor, phagocytose of IgG-opsonized sheep red blood cells, few lysosomes, dendritic shape, high levels of class II MHC molecules in their surface, and positive staining for α-manosidase and negative staining for peroxidase [1–4]. In culture, P-TR can form rosettes with immature thymocytes and modulate their proliferation [2,3]. Conversely, P-TR appear to be influenced by differentiating thymocytes [4]. Such findings raise the hypothesis that, similarly to what has been evidenced for a major component of the thymic microenvironment, namely the thymic epithelial cells, P-TR may be under intrinsic as well as extrinsic control circuits. Regarding the thymic epithelium, one relevant extrinsic group of modulators are hormones and neuropeptides [5]. Moreover, direct innervation of the organ could be envisioned as a potential modulator of intrathymic events of T cell differentiation, acting directly upon thymocytes or indirectly via microenvironmental cells. In this regard, adrenergic, cholinergic and VIPergic innervation have already been evidenced in the thymic parenchyma [6].

In non-lymphoid tissues, both norepinephrine and acetylcholine can be found co-localized with extracellular ATP (ATP_o). Actually, in the nervous system, ATP is stocked and released with norepinephrine and acetylcholine, and can act either as a neurotransmitter or as a co-transmitter [7,8]. In the endocrine system, ATP_o can act

Abbreviations: ATP_o, extracellular ATP; P-TR, phagocytic cells of the thymic reticulum.

* Corresponding author. Fax: +55 21 2808193; e-mail: pedro@chagas.biof.ufrj.br.

SSDI 0005-2736(95)00293-6

as a secretagogue for hormones as demonstrated in the pancreas and adrenals [9,10]. In the immune system, ATP_o permeabilizes the membrane of macrophages and thymic as well as peripheral lymphocytes [11–13]. It also modulates DNA synthesis, blastogenesis, and apoptosis in lymphocytes [14–16].

The effects of ATP_o are mediated by P_2 purinergic receptors. Three of them have already been cloned [17–20], but the current classification is still based on the relative order of potency of different ATP analogues and their specific responses [9,21]. Presently, five P_2 receptor subtypes have been described [9]. P_{2Y} and P_{2U} receptors act via G protein intracellular cascade, whereas P_{2T}, P_{2X}, and P_{2Z} receptors appear to act as ligand-gated channels. All of P_2 receptors are able to induce an increase of the intracellular Ca^{2+} concentration and they are differentially expressed according to the cell type. For example, P_{2T} is an ADP-selective receptor expressed in platelets, and the existence of a new ADP-selective receptor has been recently proposed in lymphocytes [22]; P_{2X} is a well characterized receptor expressed in a growing variety of excitable cell types such as neurons and muscle cells, whereas P_{2Z} is expressed in several cells of the immune-inflammatory systems, including macrophages, mast cells, thymocytes, and some peripheral lymphocyte subsets [8,9,21,23]. This receptor has also been described in some transformed cell lines and in rat parotid acinar cells [9,24].

The presence of P_{2Z} receptors can be ascertained by the activation of a cation non-selective conductance and its most striking characteristic is the permeabilization of cell membranes to low molecular weight solutes with M_r of up to 900. Furthermore ATP_o effects are mediated by ATP^{4-} ions and ATP-γ-S, but not by ADP or AMP [8,9].

In spite of several reports of ATP actions in cells of the immune system, little is known about its action on thymus physiology, and more specifically in the thymic microenvironment. Moreover, little is known about membrane receptor and signal transduction of P-TR and no information could be found about their membrane permeability and ionic channels. This prompted us to investigate the effects of ATP_o on P-TR.

2. Materials and methods

2.1. Cells

Non-epithelial phagocytic cells of the thymic reticulum (P-TR), were obtained from Swiss-Webster or Balb/c mice as previously described [1]. Briefly, the thymus was removed and minced into tiny fragments with fine scissors. These fragments were washed several times in RPMI 1640 medium to eliminate a maximum of lymphoid cells, and then incubated in complete medium (RPMI 1640 with 1% L-glutamine, 1% sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% heat-inactivated fetal calf serum), for 4 days at 37°C. The monolayers of adherent cells were then cultured for more 10–12 days until round cells with a hairy membrane profile started to proliferate and spontaneously detached from the culture plates. These cells were harvested and plated in 35 mm^2 plastic culture dishes to form P-TR-enriched secondary cultures. Experiments were performed 2–15 days later. In addition P-TR were characterized by their dendritic morphology, their phagocytic capacity, their ability to form rosettes with thymocytes, and Ia and CD11a expression [1,2,25]. More than 90% of cells had the typical morphology that allowed them to be identified under phase contrast conditions (e.g., Fig. 4).

2.2. Dye uptake assay

Cell permeabilization was assessed by observing the differential uptake of lucifer yellow ($M_r = 457$), trypan blue ($M_r = 961$) and rhodamine-dextran complex ($M_r = 38\,000$), as previously described [11]. Cells were incubated at 37°C for 10 min in PBS containing 0.5 mg/ml of lucifer yellow or rhodamine-dextran or 0.2% trypan blue either in the presence or in the absence of 5 mM ATP. Culture dishes were then washed four times using PBS and observed on a fluorescence microscope (Zeiss Axioplan) equipped with rhodamine (Zeiss BP 546/FT 580/LP 590) and fluorescein (Zeiss BP 450-490/FT 510/LP 520) filters. Photographs were taken using a constant exposure time of 30 s using a 400 ASA film (Kodak TMAX). Cell viability exceeded 95% in all assays. For each experimental condition, a minimum of 200 cells and a maximum of 700 cells were counted.

2.3. Electrophysiological measurements

Whole-cell patch clamping was performed at room temperature (23–30°C) using an EPC-7 amplifier (List Electronic, Darmstadt, Germany) according to standard techniques [26]. Gigaohm seals were formed using heat polished electrodes of 5–10 MΩ. The extracellular solution contained (in mM): 150 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$ and 10 Na-Hepes, pH 7.4 (normal extracellular solution). Unless otherwise specified, the pipette solution contained (in mM) 150 KCl, 5 NaCl, 1 $MgCl_2$, 0.01 K_2-EGTA, and 10 K-Hepes, pH 7.2 (normal intrapipette solution). Transmembrane currents and voltages were simultaneously recorded on a paper chart recorder (VII WR 3310, Graphtec Corporation, Yokohama, Japan) and in VCR tape after digitalization by a NEUROCORDER (model DR-390, Neuro Data Instruments Corporation, NY, USA).

Two different approaches were used to estimate reversal potentials of the currents under observation: (a) the holding potential was clamped at different values while stimulating the cells with several ATP_o injections, and (b) voltage ramps and voltage pulses were used during one

single ATP_o injection to generate *I–V* curves as described [27]. Both methods gave consistent and similar results.

2.4. Reagents

ATP, ADP, AMP, Adenosine, EGTA, lucifer yellow, rhodamine-dextran, RPMI 1640 culture medium, and HEPES were purchased from Sigma Chemical (St. Louis, MO, USA) whereas ATP-γ-S was from Boehringer Mannheim Biochemicals (Indianapolis, IN, USA).

2.5. Drug application

Drugs were applied to the cell surface by pneumatic and iontophoretic applications, using micropipettes similar to those usually employed for intracellular recordings (5–40 MΩ when filled with 3 M KCl) positioned at 2–20 μm from the cell surface. For pneumatic applications, pressure was manually controlled with the help of a 10 ml syringe, and the same extracellular medium used in each experiment was used as vehicle. Drug concentrations ranged from 20 μM to 10 mM, as specified in the text and figure legends, and pH was adjusted to 7.4. Iontophoretic applications were achieved by applying voltage pulses of −40 to −100 V lasting 0.1–3 s. Distilled and deionized water was used as solvent, pH was not adjusted, and, unless otherwise specified, drug concentration was 10 mM. In control experiments, none of the results here described were observed after the application of normal extracellular solution without ATP.

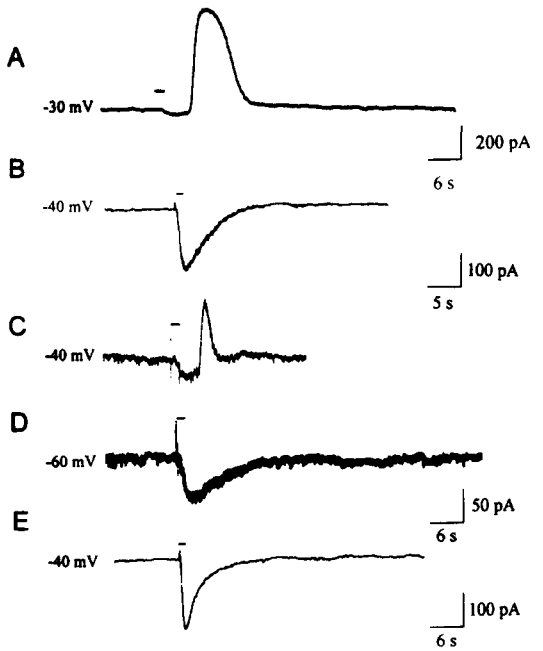

Fig. 1. Effect of extracellular ATP on membranes of P-TR. Current measurements obtained under voltage clamp by use of whole-cell patch-clamp technique. Horizontal bars indicate either pneumatic or iontophoretic ATP applications. Normal intrapipette solution was used in (A–D). In (E), the intracellular concentration of EGTA was raised to 10 mM. (A) Biphasic response induced by pneumatic application of 100 μM ATP_o 4 min after formation of whole-cell patch. (B) Inward current induced by ATP_o 23 s after formation of whole-cell patch. (C) Biphasic response induced by ATP-γ-S 57 s after formation of whole-cell patch. (D) Inward current induced by ATP-γ-S in the same cell as in (C) 114 s later. (E) Inward current induced by ATP_o 150 s after formation of whole-cell patch using an intracellular solution containing 10 mM EGTA. Holding potentials are indicated at the left side of each record.

3. Results

3.1. Currents induced by ATP_o

P-TR cells were studied using the whole-cell voltage-clamp technique ($n = 55$). At holding potentials ranging from −30 to −60 mV, both pneumatic and iontophoretic applications of ATP_o puffs in the vicinity of the cell membrane induced a transient inward current followed by an outward current in 42 cells (76%) (Fig. 1A). Some cells displayed only inward currents (18%) (Fig. 1B), while in a few cells (6%), no current could be observed. At holding potentials in the range from +30 to −90 mV, the two patterns of currents were also present but the direction of each current component changed depending on their reversal potential, as shown below. For short ATP_o applications, inward currents started right after the application, peaked at after 1–2 s and returned to zero 4–30 s later as recorded in six different experiments. When ATP_o applications were maintained for longer periods (up to 30 s) the amplitude of the inward current was sustained at peak values during the application (not shown). ATP-γ-S, a low hydrolyzable analogue of ATP, also induced biphasic responses (Fig. 1C) similar to those induced by ATP_o. Neither ADP ($n = 8$) nor AMP ($n = 4$) nor adenosine ($n = 4$), three possible ATP degradation products, induced any response in responding cells. The outward component of the response to either ATP or ATP-γ-S exhibited a fast desensitization and disappeared during the first 2–5 min following formation of the whole-cell patch (Fig. 1D). In contrast, the inward current did not show desensitization and remained active even after 15 puffs of ATP_o, applied at rate of 3 puffs/min (Fig. 1D and data not shown). When intracellular Ca^{2+} was chelated by 10 mM EGTA in the patch-pipette solution, no outward currents could be detected ($n = 8$), whereas inward currents were not abolished (Fig. 1E).

3.2. ATP^{4-} is the main form of ATP that induces membrane currents in P-TR

In order to investigate whether ATP_o-induced currents in P-TR are activated by ATP^{4-}, we first stimulated the cells by pneumatic applications of various doses of ATP_o, in normal extracellular medium. Under this condition, concentrations as low as 20 μM of ATP were able to induce biphasic or inward currents (Fig. 2A) ($n = 5$).

However, addition of 5 mM of Mg^{2+} to the extracellular medium in the same culture dish totally abolished the response (Fig. 2B) ($n = 4$). In one non-responding cell, addition of 3 mM EDTA in the extracellular medium restored the biphasic response (not shown). These results suggest that both, inward and outward currents are triggered by ATP^{4-}.

3.3. Reversal potential of the ATP_o-induced currents

In order to determine the reversal potential of the inward current ATP_o was applied to cells clamped at several holding potentials (Fig. 3) and the reversal potential obtained was near zero ($+3 \pm 3$ mV, $n = 3$). *I–V* ramps gave similar results (-2 ± 3 mV, $n = 3$) (data not shown). These results suggest that the Ca^{2+}-independent inward current may be due to a monovalent cation non-specific conductance and/or a Cl^- conductance. The reversal potential of the outward current was close to -75 mV (not shown). These data together with the dependence on the intracellular Ca^{2+} concentration suggest that this current is associated with a Ca^{2+}-dependent K^+ channels as in macrophages [27].

3.4. ATP_o permeabilizes P-TR to fluorescent dyes

The reversal potential of the inward current and its dependence on ATP^{4-} suggest the presence of P_{2Z} receptor in P-TR. To further investigate this possibility, permeabilization assays were performed. Exposure of P-TR to 5 mM ATP_o for 10 min induced permeabilization to lucifer yellow in 70% to 87% of the cells, as indicated by the presence of diffuse fluorescence inside ATP_o-treated cells (Fig. 4) but not in control cells (Fig. 4, inset). These results were obtained counting 200–700 cells in each of four independent P-TR cultures. In some control cells, lucifer yellow fluorescence was seen within peripheral cytoplasmic vacuoles characteristic of pinosomes, a pattern clearly different from the diffuse pattern induced by ATP (not shown). ATP_o was unable to induce permeabilization to the larger dyes trypan blue and rhodamine-dextran (not shown), suggesting a molecular weight cut-off smaller than 900.

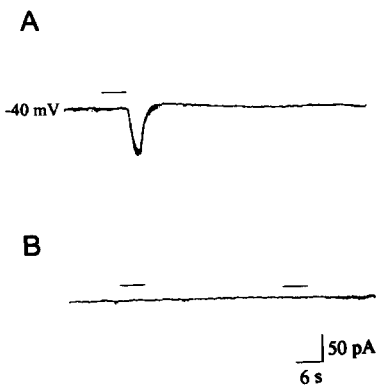

Fig. 2. Effect of extracellular Mg^{2+}. 20 μM ATP was applied pneumatically (horizontal bars). (A) Response in normal extracellular solution. (B) The same cell, 2 min after replacing the extracellular solution by one with 5 mM $MgCl_2$. Holding potential was kept at -40 mV.

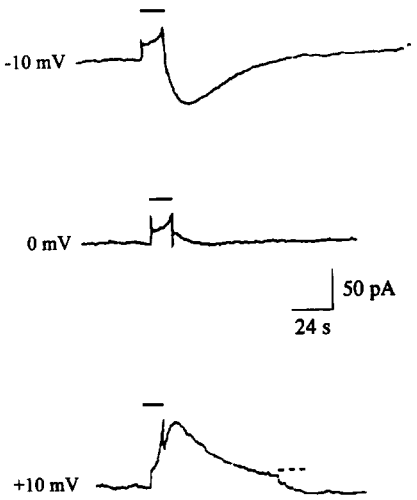

Fig. 3. Reversal potential of the inward current. Whole-cell record of the same P-TR after 3 iontophoretic applications of ATP_o (horizontal bars) at different holding potentials (V_H; -10, 0, $+10$ mV). Dashed horizontal bar in the lower trace indicate that holding potential was manually shifted from $+10$ mV to 0 mV.

4. Discussion

In recent years the presence of P_2 purinergic receptors has been reported in a variety of tissues and cells, including several bone-marrow derived cells such as macrophages, some subsets of lymphocytes, mast cells, neutrophils, megakaryocytes and multipotent hematopoietic stem cells [9]. One purinergic receptor expressed in the majority of these cells is of the P_{2Z} type. In macrophages, a ligand operated ion channel and a permeabilization phenomenon that have the properties of a pore, allow the passage of cations and molecules with M_r up to 900 [11,27]. In peripheral blood lymphocytes, thymocytes and hemopoietic stem cells, permeabilization seems to be limited to molecules of M_r below 400 [9]. This difference in molecular weight cut-off may reflect the existence of subtypes of the P_{2Z} receptors or a differential modulation of membrane permeability according to the cell type.

We investigated the effects of extracellular ATP in P-TR using two different techniques: patch-clamp current measurements and uptake of fluorescent dyes. These cells are involved in T cell maturation and retain some functional properties and surface markers of both dendritic and mononuclear-phagocytic lineage cells [1,2,4,28,29]. The patch-clamp experiments showed that when P-TR were clamped between -20 and -60 mV, ATP_o-induced currents consisted of a biphasic response: a fast inward cur-

rent followed by an outward current. The second component of the response displayed fast desensitization and usually disappeared after 2 to 5 ATP_o puffs, contrasting with the inward current that did not show desensitization.

The inward currents started right after (< 1 s) the ATP_o puff, and usually returned to zero 4–30 s after application. The fact that 10 mM EGTA in the intracellular pipette was not able to abolish the inward current indicates that it is not modulated by intracellular Ca^{2+} ions. When an ATP_o application was maintained for periods of time up to 30 s, the amplitude of the inward current was sustained. These data suggest that ATP_o exerts its effects within milliseconds, and the response is maintained as long as ATP is present in the extracellular medium at sufficient concentration. The reversal potential for the inward current was close to 0 mV, a value expected for a monovalent cation non-specific conductance or for a Cl^- conductance under our experimental conditions. The dependence of Mg^{2+} shown in Fig. 2 and the fact that ATP-γ-S is also able to elicit biphasic responses, indicate that ATP^{4-} is the main

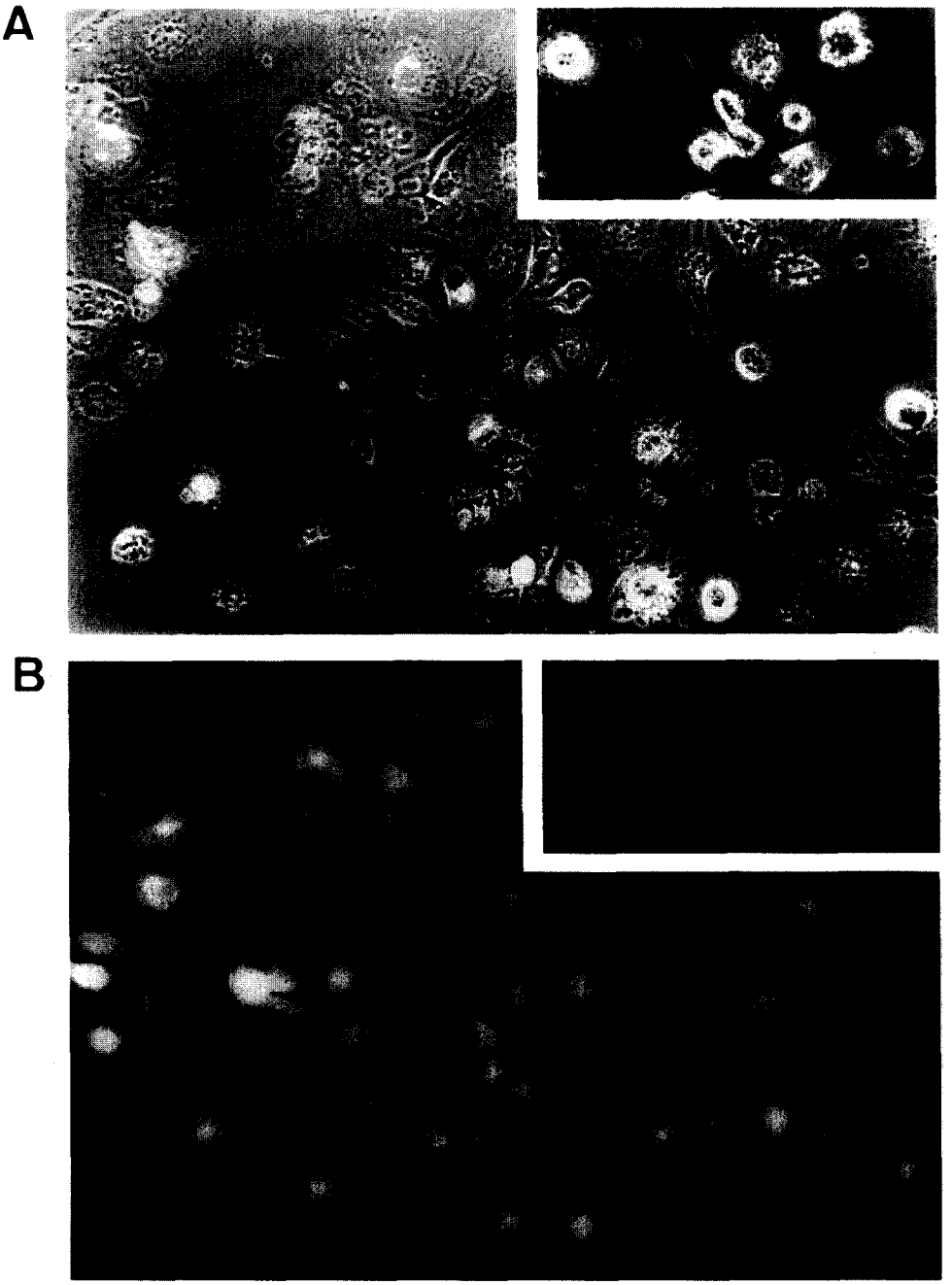

Fig. 4. ATP_o-induced permeabilization of P-TR. P-TR were plated on a culture dish and incubated in RPMI with 0.5 mg/ml lucifer yellow and 5 mM of ATP for 10 min, washed and viewed by fluorescence and phase contrast microscopy. Control cells that have been exposed to lucifer yellow and a normal extracellular solution without ATP_o are shown in the insert (upper right corner). (A) Phase contrast picture. (B) Fluorescence microscopy of the same field as in (A).

active form of ATP in P-TR. In addition these data suggest that ATP do not need to be hydrolysed to induce response. Similar results have been reported in different cells types such as macrophages [27,30,31], lymphocytes [32], mast cells [33] and thymocytes [13,34].

A further relevant aspect concerning P-TR responsiveness to extracellular ATP was that the membrane electrical changes were followed by membrane permeabilization to molecules with a M_r of at least 457 and less than 900, another characteristic of P_{2Z} receptors also present in macrophages and mast cells [9]. It should be noticed however that there is no evidence that this permeabilization phenomenon is directly associated with the inward current here described. In fact, we have recently shown that the inward current induced by ATP_o in both macrophages and P-TR are due to channels with a single channel conductance of 5–8 pS in outside-out patches of P-TR and macrophages [35]. This channel is selective for small cations and cannot explain permeability to lucifer yellow. In accordance with this view, it has been recently demonstrated that cation channels and non-selective pores can be differentially activated in *Xenopus* oocytes injected with macrophage mRNA [36]. More experiments are needed to clarify this point.

Taken together these findings indicate that P-TR express P_{2Z} receptors. However, at the moment we can not discard the presence of other kinds of P_2 purinergic receptors. Since apoptotic thymocytes and noradrenergic innervation represent possible sources of ATP_o in the thymus [7,34], it is possible that P_{2Z} receptors of P-TR may play an important role in the thymus physiology. Moreover, the similarities between P-TR and dendritic cells suggest that purinergic receptors may also be present in dendritic cells. These hypothesis is currently under investigation.

Acknowledgements

The authors dedicate this paper in honor of Prof. Carlos Chagas Filho, founder of the Institute of Biophysics, on the occasion of its 50th anniversary. We are grateful to Mariana P. Silveira and Vandir da Costa for continuous technical support, to Mr. Genilto J. Vieira for the ichnography and to Dra. Cláudia M.L.M. Coutinho for critical review of the manuscript and helpful discussions. This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq), Financiadora de Estudos e Projetos (FINEP), and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

References

[1] Papiernik, M., Nabarra, B., Savino, W., Pontoux, C. and Barbey, S. (1983) Eur. J. Immunol. 13, 147–155.
[2] El Rouby, S., Praz, F., Halbwachs-Mecarelli, L. and Papiernik, M. (1985) J. Immunol. 134, 3625–3631.
[3] Papiernik, M., Penit, C. and El Rouby, S. (1987) Eur. J. Immunol. 17, 1303–1310.
[4] Papiernik, M., Lehuen, A. and Savino, W. (1987) Cell. Immunol. 105, 280–289.
[5] Dardenne, M. and Savino, W. (1994) Immunol. Today 15, 518–523.
[6] Felten, D.L., Felten, S.Y., Carlson, S.L., Olschowka, J.A. and Livnat, S. (1985) J. Immunol. 135, 755s–765s.
[7] Von Kügelgen, I. and Starke, K. (1991) Trends Pharmacol. Sci. 12, 319–324.
[8] Burnstock, G. (1990) Ann. N.Y. Acad. Sci. 603, 1–18.
[9] Dubyak, G.R. and El-Moatassim, C. (1993) Am. J. Physiol. 265, C577–C606.
[10] Bertrand, G., Gross, R., Ribes, G. and Loubatières-Mariani, M.-M. (1990) Eur. J. Pharmacol. 182, 369–373.
[11] Steinberg, T.H., Newman, A.S., Swanson, J.A. and Silverstein, S.C. (1987) J. Biol. Chem. 262, 8884–8888.
[12] Wiley, J.S., Chen, R. and Jamieson, G.P. (1993) Arch. Biochem. Biophys. 305, 54–60.
[13] El-Moatassim, C., Bernad, N., Mani, J.-C. and Dornand, J. (1989) Biochem. Cell Biol. 67, 495–502.
[14] El-Moatassim, C., Dornand, J. and Mani, J.-C. (1987) Biochim. Biophys. Acta 927, 437–444.
[15] DosReis, G.A., Nobrega, A.F. and Carvalho, R.P. (1986) Cell. Immunol. 101, 213–231.
[16] Zheng, L.M., Zychlinsky, A., Liu, C.-C., Ojcius, D.M. and Young, J.D.-E. (1991) J. Cell Biol. 112, 279–288.
[17] Parr, C.E., Sullivan, D.M., Paradiso, A.M., Lazarowski, E.R., Burch, L.H., Olsen, J.C., Erb, L., Weisman, G.A., Boucher, R.C. and Turner, J.T. (1994) Proc. Natl. Acad. Sci. USA 91, 3275–3279.
[18] Barnard, E.A., Burnstock, G. and Webb, T.E. (1994) Trends Pharmacol. Sci. 15, 67–70.
[19] Valera, S., Hussy, N., Evans, R.J., Adami, N., North, R.A., Surprenant, A. and Buell, G. (1994) Nature 371, 516–519.
[20] Brake, A.J., Wagenbach, M.J. and Julius, D. (1994) Nature 371, 519–523.
[21] Fredholm, B.B., Abbacchio, M.P., Burnstock, G., Daly, J.W., Harden, T.K., Jacobson, K.A., Leff, P. and Williams, M. (1994) Pharm. Rev. 46, 143–156.
[22] Biffen, M. and Alexander, D.R. (1994) Biochem. J. 304, 769–774.
[23] Gordon, J.L. (1986) Biochem. J. 233, 309–319.
[24] Mcmillian, M.K., Soltoff, S.P., Cantley, L.C. and Talamo, B.R. (1987) Biochem. Biophys. Res. Commun. 149, 523–530.
[25] Alves, L.A., Carvalho, A.C.C., Lima, E.O.C., Souza, C.M.R.E., Dardenne, M., Spray, D.C. and Savino, W. (1995) Eur. J. Immunol. 25, 431–437.
[26] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflugers Arch. Eur. J. Physiol. 391, 85–100.
[27] Albuquerque, C., Oliveira, S.M., Coutinho-Silva, R., Oliveira-Castro, G.M. and Persechini, P.M. (1993) Am. J. Physiol. 265, C1663–C1673.
[28] Papiernik, M. and Homo-Delarche, F. (1983) Eur. J. Immunol. 13, 689–692.
[29] Fontecha, A.-M., Alvarez, A., Navarro, R., Zapata, A. and Ardavin, C. (1991) Immunology 73, 165–172.
[30] Buisman, H.P., Steinberg, T.H., Fischbarg, J., Silverstein, S.C., Vogelzang, S.A., Ince, C., Ypey, D.L. and Leijh, P.C.J. (1988) Proc. Natl. Acad. Sci. USA 85, 7988–7992.
[31] El-Moatassim, C. and Dubyak, G.R. (1993) J. Biol. Chem. 268, 15571–15578.
[32] Redegeld, F., Filippini, A. and Sitkovsky, M. (1991) J. Immunol. 147, 3638–3645.
[33] Tatham, P.E.R. and Lindau, M. (1990) J. Gen. Physiol. 95, 459–476.
[34] El-Moatassim, C., Mani, J.-C. and Dornand, J. (1990) Eur. J. Pharmacol. 181, 111–118.
[35] Coutinho-Silva, R., Alves, L.A., Savino, W. and Persechini, P.M. (1996) Biochim. Biophys. Acta 1278, 125–130.
[36] Nuttle, L.C. and Dubyak, G.R. (1994) J. Biol. Chem. 269, 13988–13996.