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ATP-activated Ca^{2+}-permeable channels in rat peritoneal macrophages

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The patch-clamp technique was used to study mechanisms of ATP-induced Ca^{2+} influx in rat peritoneal macrophages. The experiments on whole-cell and patch membranes have shown that extracellular ATP activates channels permeable to di- and monovalent inorganic cations. Ratios of unitary channel conductances in 105 mM Ca^{2+}, Sr^{2+}, Mn^{2+}, Ba^{2+} and normal sodium solutions were 1.0, 0.95, 0.75, 0.55 and 0.85, respectively. The channels could open in the presence of non-hydrolyzable GTP analogues in artificial intracellular solution. The data are consistent with the hypothesis that a GTP-binding protein is involved in receptor-to-channel coupling.

Macrophage; ATP; Patch clamp; Calcium channel

1. INTRODUCTION

Macrophages play an important role in host defense against infective agents and neoplastic cells. Defense responses are triggered and regulated by numerous extracellular signalling agents, such as immunoglobulins, growth factors, lymphokines etc. [1]. One of the earliest events in the stimulus–response coupling in macrophages, as in many other cell types, is Ca^{2+} influx from the extracellular medium [2–5]. Although this phenomenon has attracted a great deal of interest there is no experimental basis for the understanding of mechanisms of receptor-mediated Ca^{2+} influx.

One of the agonists known to induce a Ca^{2+} signal in macrophages is ATP in its anionic form [3,6]. Here we report a patch-clamp study of a Ca^{2+} pathway activated by ATP in the plasma membrane of rat macrophages.

2. MATERIALS AND METHODS

Peritoneal macrophages from male Wistar rats were obtained essentially as described in [7]. The culture was maintained in DMEM supplemented with 10% fetal bovine serum, 1,000 U/ml penicillin, 100 μg/ml streptomycin in a 5% CO_2 humidified atmosphere.

Patch-clamp experiments were performed essentially as described earlier [8] at 22–24°C.

The control cytosol-like solution contained (in mM): K_2SO_4 80; CsCl 10; HEPES-KOH 10; EGTA-KOH 10; $MgCl_2$ 2; $CaCl_2$ 0.5 (pH 7.3; pCa ≥ 8.0). The control external solution contained (in mM): NaCl 145; KCl 5; Tris-HCl 10; $CaCl_2$ 2; $MgCl_2$ 1 (pH 7.4). Isotonic divalent cation solutions contained 105 mM $MeCl_2$ plus 10 mM Tris-HCl. When calculating free ATP (ATP^{4-}) concentrations apparent p*K*s were taken to be 3.98 and 4.32 for Ca^{2+} and Mg^{2+}, respectively [9]. For Ba^{2+} the pK was taken to be equal to 3.98.

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Abbreviations: ATP, adenosine triphosphate; DMEM, Dulbecco's modified Eagle's medium; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid; GTP, guanosine triphosphate; GTPγS, 5′-*O*-(3-thiotriphosphate).

3. RESULTS AND DISCUSSION

ATP was used at total concentrations from 50 to 500 μM with ATP^{4-} concentrations of no more than 5 μM in order to avoid the development of non-selective membrane conductance [3,10]. Fig. 1 shows a whole-cell inward current through a macrophage membrane elicited by ATP when the bath solution contained 105 mM $BaCl_2$. The current reached a maximal value within several seconds of ATP addition and then began to decline. It can be seen that the ATP-induced current is much noisier than the respective basal current, suggesting that it flows through the channels which open and close in a step-like manner. At the beginning of the response the resulting noise was too large for unitary current levels to be resolved (trace 2 in Fig. 1B) but 30 s later, when the whole current decreased to approximately 25% of its peak value, current levels of about 1.0 pA could be discerned. Still later, separate pulse-like currents of 1.0 pA could be seen (traces 4 and 5 in Fig. 1B). Rare events of the same amplitude were seen before ATP application, indicating that the channels could sometimes open spontaneously. The unitary currents at potentials from −60 to −30 mV form a current–voltage relation with a slope conductance of 11 pS and an extrapolated current reversal potential at about +40 mV. A positive value of reversal potential suggests that currents were carried by Ba^{2+} (the major cation in the external solution) rather than internal anions (see Section 2) going in the opposite direction. Similar unitary current–voltage relations

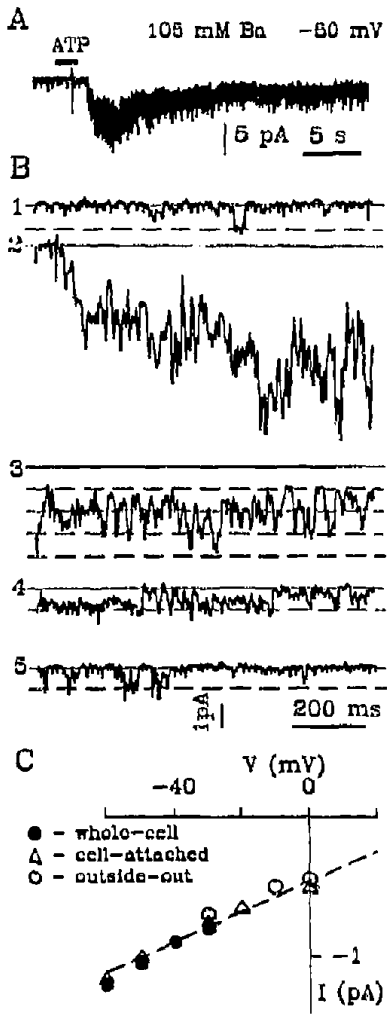

Fig. 1. Integral and unitary currents activated by ATP. Whole-cell configuration, 105 mM $BaCl_2$ in the bath solution, ATP is applied at 500 μM (ATP^{4-} concentration is 0.5 μM). (A) Integral current at −50 mV before and after the ATP addition. (B) Records from the same experiment as in A on expanded current and time scales before (1) and 2 (2), 30 (3), 76 (4) and 220 (5) s after ATP addition. The solid line indicates the basal current level. (C) Unitary current–voltage relations for ATP-activated channels with 105 mM Ba^{2+} as charge carrier. Filled circles indicate currents from the experiment shown in A and B. Open triangles and circles indicate currents from individual cell-attached (representative of 13) and outside-out (representative of 5) experiments, respectively.

were resolved in three more whole-cell experiments. Activity of the same channels, judging by current amplitudes, were also seen in cell-attached and outside-out experiments (Fig. 1C).

In cell-attached experiments channel activity was seen almost exclusively when ATP was added to the solutions adjacent to the extracellular surface of the patch membrane (13 experiments). Thus, the channels in on-cell patches seemed to be activated by ATP just like as in whole-cell membrane.

When working in the outside-out configuration, GTPγS, a non-hydrolyzable analogue of GTP, was added to the internal pipette solution. GTPγS seems to

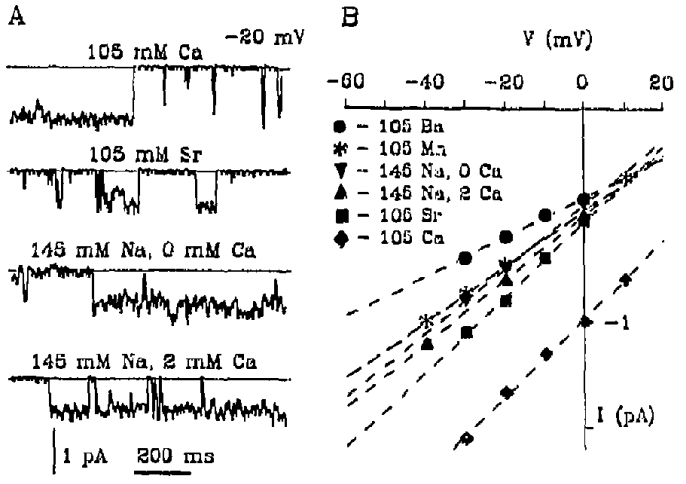

Fig. 2. Selectivity of ATP-activated channels. (A) Current records at −20 mV obtained in one individual outside-out experiment with 105 mM $CaCl_2$, $SrCl_2$, sodium Ca-free and normal solutions. (B) Unitary current–voltage relations for 105 mM $BaCl_2$, $MnCl_2$, $SrCl_2$, $CaCl_2$ and sodium Ca-free and normal solutions. Each point is the mean of 2–8 outside-out experiments.

be essential for channel activity to occur without any agonist application on the external surface of the membrane. Indeed, in 6 (of 10) inside-out experiments, GTPγS (100 μM) applied to the intracellular side of the membrane increased the channel activity. The effect of GTPγS raises the possibility that the channel activation is mediated by G proteins [11]. This is in agreement with the evidence for the involvement of a pertussis-sensitive G protein in the development of ATP-induced Ca influx in macrophages [6].

Fig. 2A illustrates selective properties of the channels. Among the divalent cations tested, current amplitudes at potentials from −30 to 0 mV were in the following succession: $Ca^{2+} > Sr^{2+} > Mn^{2+} > Ba^{2+}$. Current–voltage relations shown in Fig. 2B have conductances (in pS) of 20, 19, 15 and 11 for 105 mM of Ca^{2+}, Sr^{2+}, Mn^{2+}, Ba^{2+},

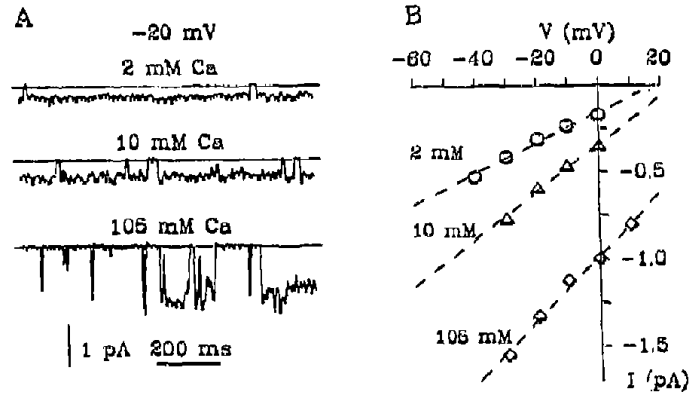

Fig. 3. The dependence of unitary currents on Ca^{2+} concentration. (A) Currents in an outside-out patch at −20 mV with 2, 10 and 105 mM $CaCl_2$ in the bath solution. The normal tonicity of the solutions was maintained by the addition of Tris-HCl. (B) Unitary current–voltage relations with 2, 10 and 105 mM $CaCl_2$. The points on the plot are the mean of 2–6 experiments.

respectively. Two more levels of current amplitudes and respective conductances differing from that shown in the figures by factors of approximately 0.5 and 1.5 were also observed. Currents and unitary conductances in normal sodium solution were intermediate between those for isotonic Sr^{2+} and Mn^{2+}. Substitution of all inorganic cations by $Tris^{+}$ abolished currents completely (not shown), indicating channel impermeability to this organic cation. Thus, the channels are highly permeable to small metal mono- and divalent cations, with permeability for Ca^{2+} being essentially higher than for the other cations tested.

Single-channel currents at 2, 10 and 105 mM Ca^{2+} are shown in Fig. 3. Currents at 40 mM Ca^{2+} (not shown) differed from those at 105 mM only slightly, thus Ca^{2+} flux through the channel saturates at concentrations higher than 40 mM. Slope conductance with a physiological concentration of Ca^{2+} (2 mM) is about 10 pS, only two times lower than that with 105 mM.

Whole-cell currents in 2 mM Ca^{2+} solution evoked by ATP (ATP^{4-} concentrations of 2.5 or 5.0 μM) were from 1.5 to 60 pA (mean 20 pA, 15 experiments) which seems to be good enough to raise the cytosol Ca^{2+} concentration to near micromolar levels [3,6].

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