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Activation of single Ca^{2+}-dependent K^+ channel by external ATP in mouse macrophages

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Single Ca^{2+}-dependent K^+ currents responding to external ATP were recorded from cell-attached patches on mouse peritoneal macrophages. Extracellularly applied ATP activated an inward single-channel current with a conductance of 25 pS and a reversal potential of −79 mV (pipette potential, V_p) when the pipette contained a 145 mM KCl solution. The reversal potential was shifted 56 mV positive by a 10-fold reduction in external (pipette) K^+ concentration. The effect of ATP was abolished by either removal of external Ca^{2+} or treatment with an intracellular Ca^{2+} chelator, the acetoxymethyl ester of 1,2-*bis* (2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA-AM). This channel has a mean open time of 9.1 ms and open probability was not strongly dependent on V_p in the range tested (+120 to −30 mV).

External ATP; Ca^{2+}-dependent K^+ conductance; Single channel; Patch-clamp; Mouse peritoneal macrophage

1. INTRODUCTION

Several recent studies in macrophages have shown that external ATP induces an increase in $[Ca^{2+}]_i$ [1,2], depolarization of plasma membrane via the activation of ion nonselective conductance [1,3], and inhibition of Fc receptor-mediated phagocytosis [1]. Since ATP is released from platelets [4], endothelial cells [5] and neurons [6] under certain situations, ATP may serve as an intercellular messenger in regulating physiological function of macrophages.

Recently, we have demonstrated, using the perforated patch-clamp recording in mouse macrophages, that ATP activates a Ca^{2+}-dependent K^+ channel via an influx of Ca^{2+} across the plasma membrane, resulting in membrane hyperpolarization [7]. The activation of this channel by ATP was completely blocked by quinidine, a known K^+ channel blocker [7]. On the other hand, quinine has been reported to inhibit phagocytosis by mouse macrophages [8], suggesting an important role of K^+ channel in macrophage function. There are at least three types of Ca^{2+}-dependent K^+ channels in a variety of tissues (large, intermediate, or small conductance) [9]. These observations prompted us to evaluate which type of Ca^{2+}-dependent K^+ channel is activated by ATP in macrophages for understanding the mechanism underlying phagocytosis. Using single-channel recording technique, we clearly demonstrate that ATP activates a Ca^{2+}-dependent K^+ channel which has the intermediate conductance and whose opening is not strongly dependent on the membrane potential. It appears that this conductance may be important in regulating macrophage functions such as phagocytosis and adhesion.

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2. MATERIALS AND METHODS

Mouse peritoneal macrophages were collected and cultured as described previously [7]. Coverslips with adherent cells were mounted in a recording chamber (0.3 ml) and perfused at a rate of 1 ml/min with standard bath solution containing (in mM): 145 NaCl, 4.5 KCl, 1.6 $CaCl_2$, 1.13 $MgCl_2$, 10 Hepes, pH 7.2. Single-channel currents were recorded by the cell-attached patch-clamp method using a List EPC-7 patch-clamp amplifier [10]. Unless otherwise stated, patch pipettes (2–5 MΩ) were filled with the 145 mM KCl solution containing (in mM): 145 KCl, 1 $MgCl_2$, 0.1 $CaCl_2$, 1.1 EGTA, 10 Hepes, pH 7.2. In some experiments, K^+ concentration in the pipette was varied by replacing KCl with an equimolar concentration of NaCl. Single-channel data were stored on an FM tape recorder for later analysis. Currents were later filtered at 1 kHz (−3 dB, 24 dB/octover), digitized at 2 kHz using Canopus 12-bit A/D converter and analyzed with a NEC PC-9801 personal computer. The mean open probability, P_o, was calculated from the area under peaks of the amplitude histogram after fitting each peak with a Gaussian curve. The mean open time was calculated from the open time histogram by a single exponential fitting. ATP was extracellularly applied by means of a pressure ejection system (PPM-2 Medical System) [7]. ATP (sodium salt) was purchased from Boehringer and BAPTA-AM from Dojin.

3. RESULTS AND DISCUSSION

After a delay of 1–2 s, application of ATP (100 μM) elicited discrete channel activity with simultaneous opening of up to five channels on the patch held at a potential (V_p) of +60 mV (Fig. 1A). Positive V_p corresponds to hyperpolarization from the resting poten-

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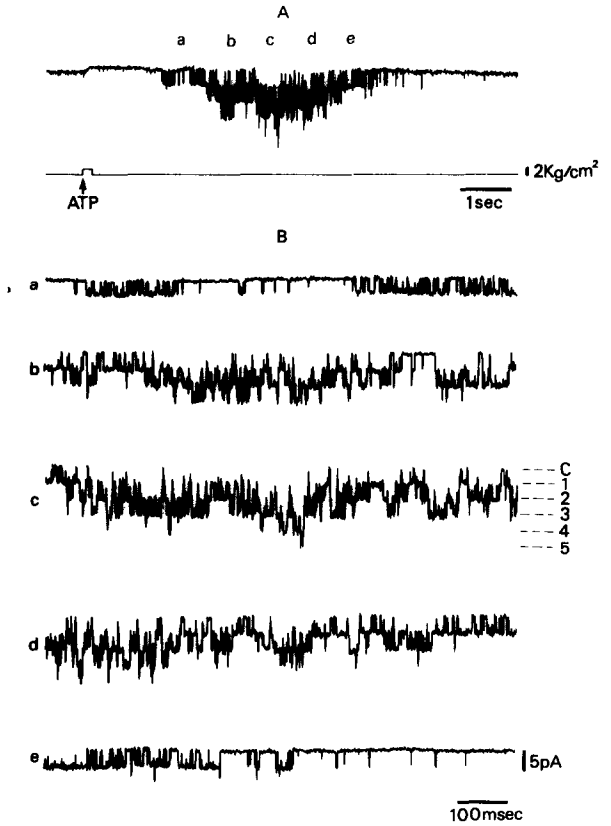

Fig. 1. (A) ATP activation of a single-channel current in cell-attached patch. The patch is held at +60 mV. ATP (100 μM) was applied at times indicated in the lower traces in this and subsequent figures. (B) Expanded current traces of record in (A) indicated as a–e. Inward current activity is shown by deflections below the baseline as indicated by C (closed state) and open channel levels are indicated to the right of traces by *numbers* in this and subsequent figures.

tial. Since ATP cannot diffuse from the bathing medium into the area of the patched membrane, activation of these channels should be indirect, presumably via a diffusible second messenger(s).

To determine the channel conductance and ion specificity, the patch was held at a various V_p during the ATP-induced responses (corresponding to part c in Fig. 1A) (Fig. 2A). Current-voltage relations revealed that the channel shows slightly inward rectification with a conductance of 25 pS and a reversal potential of −79 mV (145 mM KCl, in the pipette) (Fig. 2C). Since intracellular K^+ concentration is approximately 160 mM [1], the predicted reversal potential for a K^+ current across the patch membrane is about −2 mV. The reversal of current at −79 mV implies the membrane potential to be −77 mV. This is consistent with our previous observation that external ATP hyperpolarized macrophages to −80 mV [7]. With use of low K^+ (36 mM KCl) in the pipette, the channel has a slope conductance of 15 pS and a reversal potential of −45 mV (Fig. 2B, C). The shift in the reversal potential was 34 mV, close to the calculated value of 35 mV for K^+. Therefore, K^+ is the main ionic species carrying the ATP-induced current.

As shown in Fig. 3A and B, either the removal of external Ca^{2+} (0.1 × normal Ca^{2+}) or the bath application of BAPTA-AM (50 μM), a membranepermeable and highly specific chelator for Ca^{2+} [11], almost completely abolished the ATP-induced channel activity. Thus, the action of ATP is probably mediated by an increase in $[Ca^{2+}]_i$ resulting from Ca^{2+} influx across the plasma membrane.

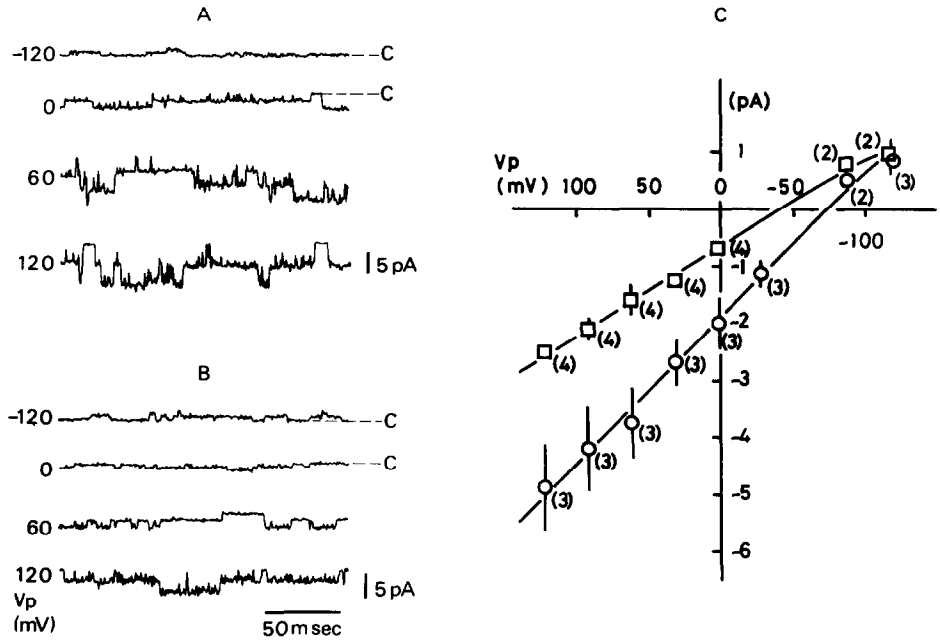

Fig. 2. Conductance and ion selectivity of the ATP-induced single-channel currents. (A, B) Expanded current records corresponded to part c of Fig. 1A with the symmetrical 145 mM KCl (A) or 36 mM KCl (B) pipette solution at different V_p (indicated to the left). (C) I–V relations from (A) (open circles) and (B) (open squares). Data points are mean ± SD. Number of patches used to determine each point is shown in parentheses.

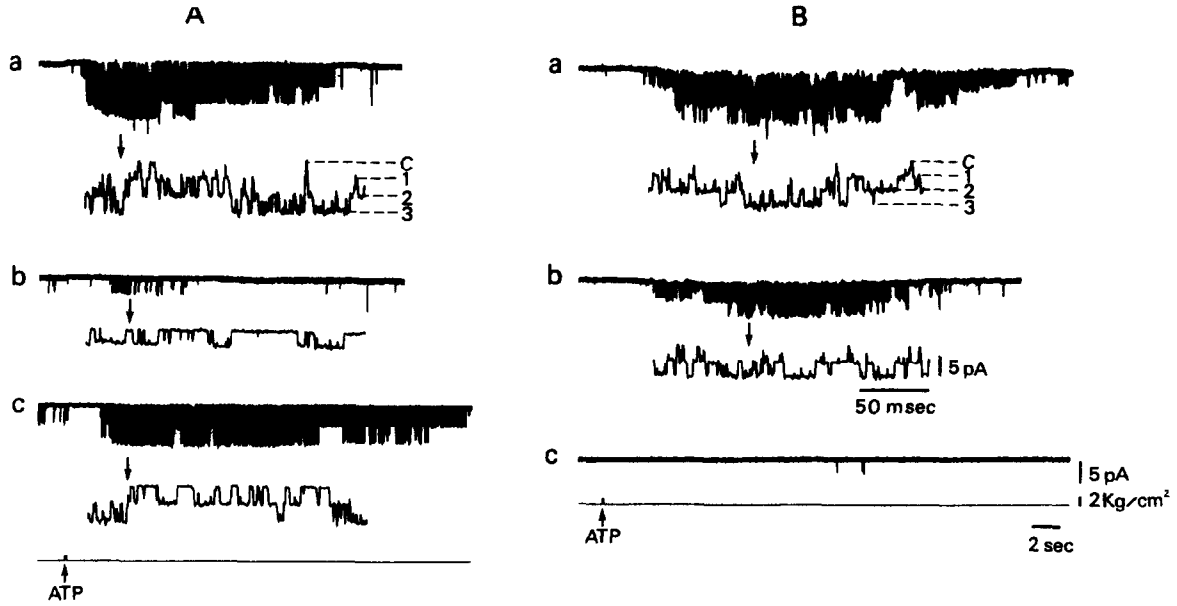

Fig. 3. Effects of removal of external Ca^{2+} (A) and 50 μM BAPTA-AM (B) on the ATP-induced response. (A) a, control; b, 6 min after exposure to low Ca^{2+} (0.1 × normal Ca^{2+}); c, 4 min after washout. (B) a, control; b and c, 2 min and 4 min after exposure of 50 μM BAPTA-AM. V_p was held at +60 mV. Inserts below traces present segments of data on a faster time scale.

In Fig. 4A, the observed values for the probability to find 0, 1, 2,... N channels in the open state were compared with the predicted value calculated from the binomial distribution. A good agreement between observed and predicted values was found. Thus, the ATP-induced channels have similar properties and act independently of one another. The open time histogram of the channel could be fitted by a single exponential curve with a time constant of about 10 ms in the patch where only one channel was recorded. The averaged mean open time at V_p of +60 mV was 9.1 ± 2.0 ms (mean ± SD, n = 5) and did not show a clear voltage-dependency (data not shown). P_o also showed little dependence on membrane potential over the voltage range tested (Fig. 4C).

The Ca^{2+}-dependent K^+ conductance described here closely resembles the channel activated by ionomycin in human macrophages (36 pS in symmetrical KCl) [12] and the channel activated by ionomycin or mitogenic lectin in rat thymocytes (25 pS in symmetrical KCl) [13,14] in their conductances and I–V relations.

The binding of IgG-coated erythrocytes to mouse macrophage Fc receptor results in a transient increase in $[Ca^{2+}]_i$ and triggers phagocytosis [8]. Such an in-

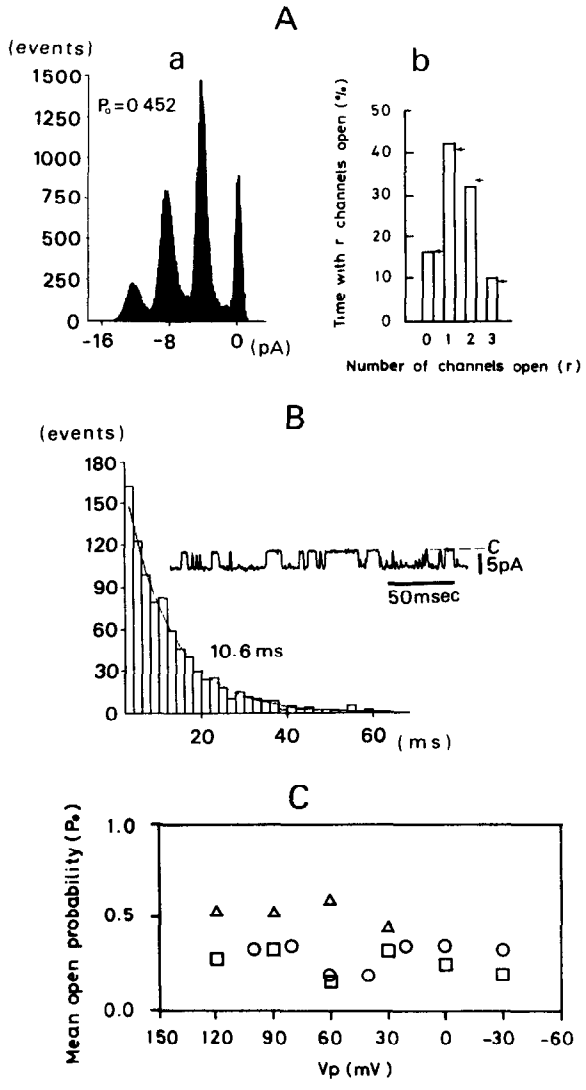

Fig. 4. Kinetic analysis of the ATP-induced single-channel current. (A) a, amplitude histogram of single channel events; b, percentage of time during which zero, one, two or three channels (r) were simultaneously open during the ATP-induced response. Observed values and predicted values calculated from the binomial distribution are represented by open columns and arrows, respectively. V_p was held at +60 mV. (B) Open time histogram at V_p of +60 mV. Insert shows original current record on an expanded time scale. (C) Voltage-dependency of mean open probability (P_o). Open triangles, open squares and open circles represent three different patches.

crease in $[Ca^{2+}]_i$ in turn activates a Ca^{2+}-dependent K^+ conductance, resulting in membrane hyperpolarization [15]. Quinine, a known K^+ channel blocker, inhibits phagocytosis in these cells [8]. Moreover, in B and T lymphocytes, mitogenic lectin and antibodies activate Ca^{2+}-dependent K^+ conductances during their activations [16,17]. Therefore, we speculate that the complex cellular function of phagocytosis and adhesion in macrophages may be initiated via the activity of a Ca^{2+}-dependent K^+ conductance as well as a Ca^{2+} conductance. However, further investigation will be necessary to determine the precise physiological function of these events in the activation of immune cells.

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