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Selectivity of ATP-activated GTP-dependent Ca^{2+}-permeable Channels in Rat Macrophage Plasma Membrane

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Abstract. Outside-out configuration of the patch clamp technique was used to test whether an intracellular application of G protein activator (GTPγS) affects ATP-activated Ca^{2+}-permeable channels in rat macrophages without any agonist in the bath solution. With 145 mM K^+ (pCa 8.0) in the pipette solution, activity of channels permeable to a variety of divalent cations and Na^+ was observed and general channel characteristics were found to be identical to those of ATP-activated ones. Absence of extracellular ATP makes it possible to avoid the influence of ATP receptor desensitization and to study the channel selectivity using a number of divalent cations (105 mM) and Na^+ (145 mM) as the charge carriers. Permeability sequence estimated by extrapolated reversal potential measurements was: Ca^{2+} : Ba^{2+} : Mn^{2+} : Sr^{2+} : Na^+ : K^+ = 68 : 30 : 26 : 10 : 3.5 : 1. Slope conductances (in pS) for permeant ions rank as follows: Ca^{2+} : Sr^{2+} : Na^+ : Mn^{2+} : Ba^{2+} = 19 : 18 : 14 : 12 : 10. Unitary Ca^{2+} currents display a tendency to saturate with the Ca^{2+} concentration increase with apparent dissociation constant (K_d) of 10 mM. No block of Na^+ permeation by extracellular Ca^{2+} in millimolar range was found. The data obtained suggest that (i) activation of some G protein is sufficient to gate the channels without the ATP receptor being occupied, (ii) the ATP receptor activation results in the gating of a special channel with the properties that differ markedly from those of the receptor-operated or voltage-gated Ca^{2+}-permeable channels on the other cell types.

Key words: ATP receptor — G protein — Patch clamp — Ca^{2+}-permeable channels — Rat macrophages

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Introduction

ATP-activated channels have been reported in a variety of cells such as smooth muscle cells (Benham & Tsien, 1987; Nakazawa & Matsuki, 1987; Friel, 1988), cardiac cells (Friel & Bean, 1988) sensory neurons (Krishtal et al., 1988), pheochromocytoma cells (Nakazawa et al., 1990*a;* Neuhaus, Reber & Reuter, 1991; Vincent, 1992) and macrophages (Naumov et al., 1992; Alonso-Torre & Trautmann, 1993). Divalent as well as monovalent cations are permeable through these channels. Permeability for Ca^{2+}, estimated from reversal potentials of macroscopic currents under whole-cell recording conditions, in smooth muscle cells and in pheochromocytoma cells is several times greater than that for Na^+ (Benham & Tsien, 1987; Nakazawa et al., 1990*a*). Nevertheless, unitary Ca^{2+} currents are much smaller than the Na^+ ones (Benham & Tsien, 1987; Sasaki & Gallacher, 1990; Nakazawa et al., 1990*b*). The inhibitory effect of external Ca^{2+} on sodium currents has been shown at the single-channel level (Nakazawa et al., 1990*a,b;* Neuhaus et al., 1991; Nakazawa & Hess, 1993). The results suggest that Ca^{2+} might compete with Na^+ at a binding site in the channel pore and may be explained qualitatively using the single-file multi-ion model of L-channels (Hess & Tsien, 1984; Almers & McCleskey, 1984). ATP-activated single channels with rather high selectivity to Ca^{2+} *vs.* K^+ (P_{Ca}/P_K = 71) estimated from extrapolated reversal potential values have been described in our recent study on rat macrophages (Naumov et al., 1995*a*). The aim of the present study is to investigate the ATP-activated channel selectivity more systematically using a number of divalent cations and Na^+ as current carriers. GTP or GTPγS application to the intracellular surface of the patch mimics the effect of ATP that indicates involvement of GTP proteins in the transduction of a signal from the ATP receptor to the channel (Naumov et al., 1995*b*). This fact has allowed us to perform experiments

in outside-out configuration of the patch clamp technique with GTPγS added to the pipette solution without ATP in the bath. The results indicate the general characteristics of currents recorded in these conditions to be identical to those of ATP-activated channels. The permeability ratios for divalent *vs.* monovalent ions estimated by reversal potential and slope unitary conductance measurements reveal significantly different selectivity sequences. The data obtained make it evident that ATP-activated channels in rat macrophages differ from yet known Ca^{2+}-permeable channels in other cell types.

Materials and Methods

Cells

Peritoneal resident macrophages from male Wistar rats (5–10-weeks old) were obtained essentially as described before (Conrad, 1981). The cells on coverslips were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 mg/ml streptomycin in the 5% CO_2 humidified atmosphere for 1–10 days at 37°C. Cultured macrophages were used in patch clamp experiments 2–10 days after plating. Cells were identified as macrophages with the use of α-naphthyl acetate esterase staining (Monahan, Dvorak & Dvorak, 1981). It was established that no less than 98% of cells in monolayers were macrophages. The identification was carried out in parallel with patch experiments.

Patch Clamp Method

Ionic currents were studied in the outside-out configurations of the patch clamp technique (Hamill et al., 1981). Micropipettes were made from soft glass, covered with Sylgard 184, fire polished immediately before use and had a resistance of 8–12 *M*Ω. The pipette with the excised membrane fragment was transferred to a smaller chamber (about 20 μl). The bath was grounded with the Ag-AgCl electrode separated from the bathing solution by a short Ringer-agar bridge, the potential inside the pipette was clamped at the required level. In the beginning of the experiment, offset potentials in the voltage recording system were adjusted to give zero current between the pipette solution and the bathing solution. No correction for the liquid junction potential was performed. Current signal was measured with the patch clamp amplifier with a conventional resistive feedback in the headstage 20 GΩ, was recorded at a bandwidth 1.2 or 2.5 kHz using an analog FM recorder and stored on a magnetic tape. Data, filtered by 6-pole low-pass Bessel filter at a cutoff frequency 0.3 kHz, were digitized at 1 msec/pt on A/D converter PCL-718 and analyzed offline with IBM-compatible computer.

Measurements of the unitary current amplitudes were performed by either of two methods. In the first method, the unitary current was calculated by fitting with Gaussian functions for the distribution of current amplitude. In the second method, the difference between the averaged current amplitude during long channel opening and the baseline was considered to be the unitary current for that opening. In both methods, the current amplitudes corresponded to the third current sublevel detected in the study of ATP-activated channels (Naumov et al., 1995*a*).

Averaged data are given as the mean + SEM (number of experiments). Experiments were carried out at a room temperature 23–24°C.

Solutions

Internal pipette solution contained (mM): 135 KCl, 1 $MgCl_2$, 10 HEPES/KOH, 2 EGTA/KOH, 0.2 $CaCl_2$ (pCa 8, pH 7.4). To eliminate activity of Cl^- channels, chloride ions in the pipette solution were substituted with aspartate or glutamate in some experiments. No differences in ATP-activated channel characteristics were detected whether Cl^- or organic anions were used or not, and no activity of Ca-dependent potassium of Cl^- channels was detected in similar ionic conditions. External solutions contained 105 mM $CaCl_2$ (or $BaCl_2$, $SrCl_2$, $MnCl_2$) and 10 mM Tris-HCl (pH 7.4). Isomolarity of solutions with Ca^{2+} concentrations less than 105 mM was maintained by addition of Tris $^+$. Whole-cell recording experiments (Naumov et al., 1995*a*) demonstrated impermeability of ATP-activated channels to $Tris^+$. Solution with 145 mM NaCl plus 10 mM Tris-HCl was also used. Solutions with different cation concentrations were applied to patches either by bath perfusion or by brief pressure ejection. In both cases, the duration of the complete change in the solution around the cell was <300 msec.

GTP and GTPγS were obtained from Sigma; EGTA from Fluka.

Results

Identification of ATP-activated Channels

Properties of ATP-activated Ca^{2+}-permeable channels in the rat macrophage plasma membrane were described earlier (Naumov et al., 1995*a*). Investigations were performed using cell-attached and outside-out configurations of the patch clamp method. Channel activity in cell-attached experiments was observed when the pipette solution contained 100 μM ATP in 68% of all attempts (n = 60). No activity was detected in absence of ATP (n = 19). The activity lasted for 30–40 sec and then disappeared almost completely, probably due to desensitization of the ATP receptor. Patch excision was followed by rapid disappearance of currents. Subsequent addition of GTP or GTPγS to the internal membrane surface resumed the activity (Naumov et al., 1995*b*) with the delay averaged 21 ± 8 sec (n = 18). In experiments without an agonist in the pipette solution, the same results were obtained but the delay in the activity appearance increased up to 4 min (n = 5). The difference in the delay is consistent with prolonged coupling of GTPγS with the effector when the receptor is not occupied with an agonist. Resumption of the channel activity was successful in 49% of the patches investigated (n = 69). An example of a typical experiment performed in cell-attached and then in inside-out configurations is presented in Fig. 1. The pipette solution contained 105 mM Ba^{2+} as the only permeate cation and 100 μM ATP. The averaged current-voltage relations presented in Fig. 1*C* give the slope conductance (γ) and extrapolated reversal potential (V_r) of about 10 pS and +40 mV. Similarity of averaged current-voltage curves obtained in both configurations indicates that application of GTP or GTPγS induces the ac-

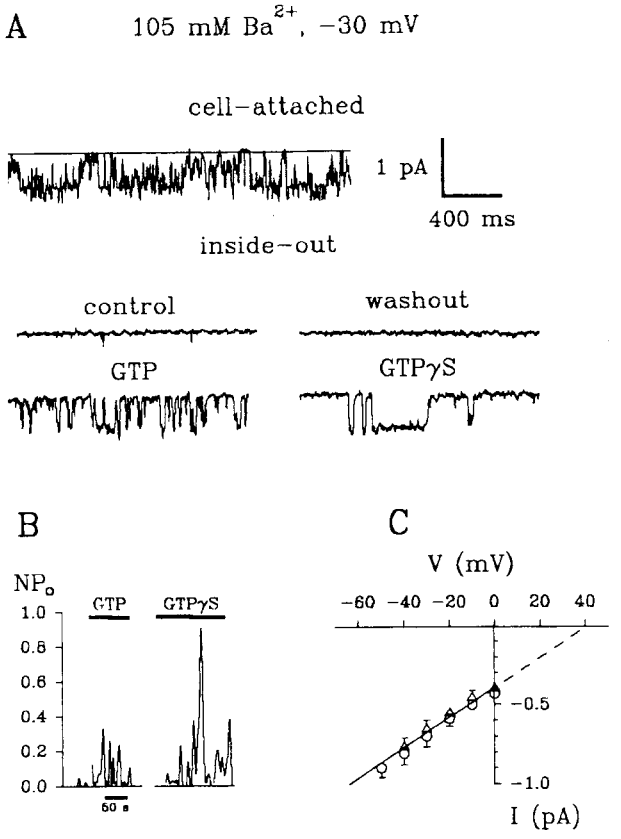

Fig. 1. Identity of ATP-activated and GTP-induced Ca-permeable channels in rat macrophages plasma membrane. (*A*) An example of current records at −30 mV obtained in typical experiment in cell-attached (top) and inside-out configurations (bottom). Pipette solution contained 105 mM Ba^{2+} and 100 μM ATP. Patch excision was followed by rapid disappearance of currents (control) and greatly augmented after the addition of 100 μM GTP to the cytosol-like solution containing 145 mM K^+ and 2 mM EGTA (pCa 8). Washout of GTP resulted in a complete disappearance of inward currents. Subsequent addition of 100 μM GTPγS resumed the channel activity. (*B*) Time course of the channel activity after the application of GTP or GTPγS to the internal membrane surface estimated as $NP_o = \langle I \rangle / i$, where N is the number of open channels, P_o—open probability, $\langle I \rangle$—mean channel current and i—unitary current amplitude. (*C*) Averaged current amplitudes *vs.* membrane potential collected from 3–5 experiments in cell-attached mode with 100 μM ATP in the pipette solution (circles) and from 6–8 experiments in inside-out configuration after the addition of GTP into intracellular solution (triangles). Error bars denote SEM. The current amplitudes correspond to the most frequent 3rd current sublevel (Naumov et al., 1995*a*).

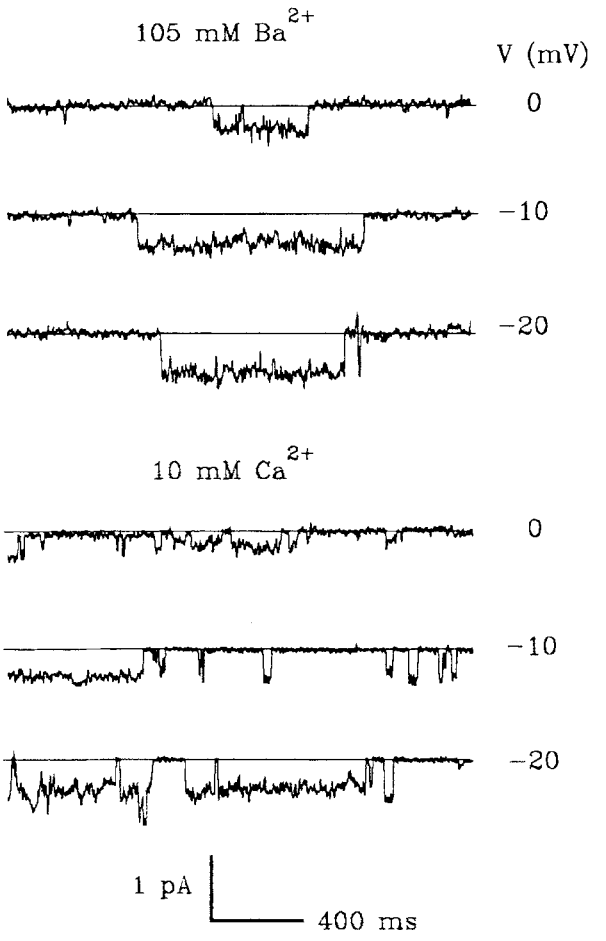

Fig. 2. Single-channel activity in outside-out patch in the presence of 100 μM GTPγS in the pipette solution at different holding potentials. Solid lines indicate the closed state of the channel. Intracellular solution contained 145 mM K^+, (with glutamate as the main anion), 2 mM EGTA (pCa 8); extracellular solution contained 105 mM Ba^{2+} (top traces) or 10 mM Ca^{2+} (bottom traces). Holding membrane potentials are indicted near current traces.

tivity of the same channels that are activated by ATP in cell-attached mode.

As application of GTPγS to the internal membrane surface mimicked the effect of extracellular ATP on the channel activity one could expect that GTPγS only being introduced in the pipette solution would activate these channels in outside-out patches. Thus, in the present study, experiments on outside-out excised patches were carried out with 100 μM GTPγS added to the intrapipette solution. Channel activity was observed in approximately 70% of experiments (n = 45) without the ATP application to the bath solution. With GTPγS in the pipette solution, outside-out patches occasionally were less stable than the cell-attached and inside-out ones, therefore the current amplitude measurements were bordered by the narrow range of negative potentials. The absence of ATP provided for the elimination of the receptor desensitization process. The experiment could be long enough (up to 15 min) to allow the current registration with different ionic content solutions applied to the external surface of the patch. Application of 100 μM ATP to the bath had not effect on the GTPγS-induced channel activity in outside-out patches (n = 5).

Figure 2 shows the current records from an excised outside-out patch when intracellular solution contained 145 mM K^+ (with glutamate as the main anion), 2 mM EGTA (pCa = 8) and 100 μM GTPγS and extracellular

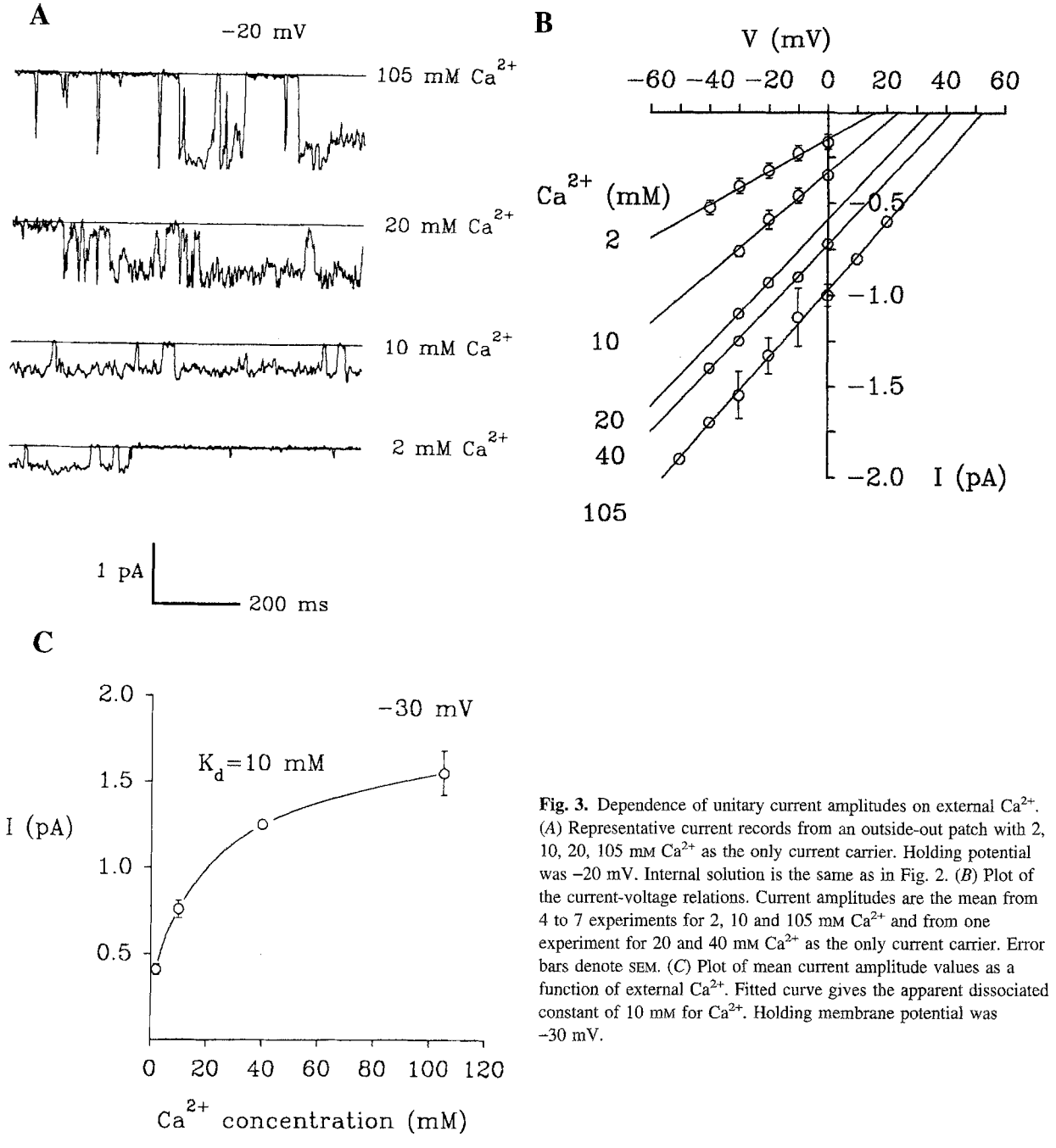

Fig. 3. Dependence of unitary current amplitudes on external Ca^{2+}. (*A*) Representative current records from an outside-out patch with 2, 10, 20, 105 mM Ca^{2+} as the only current carrier. Holding potential was −20 mV. Internal solution is the same as in Fig. 2. (*B*) Plot of the current-voltage relations. Current amplitudes are the mean from 4 to 7 experiments for 2, 10 and 105 mM Ca^{2+} and from one experiment for 20 and 40 mM Ca^{2+} as the only current carrier. Error bars denote SEM. (*C*) Plot of mean current amplitude values as a function of external Ca^{2+}. Fitted curve gives the apparent dissociated constant of 10 mM for Ca^{2+}. Holding membrane potential was −30 mV.

solutions containing 105 mM Ba^{2+} or 10 mM Ca^{2+}. The averaged current-voltage relations presented in Figs. 3*B* and 5 give γ and V_r values identical to those of ATP-activated channels recorded in cell-attached and outside-out modes (Naumov et al., 1995*a,b; see also* Fig. 1) in similar ionic conditions. For 105 mM Ba^{2+} (Fig. 5), γ and V_r were equal to 10 pS and +43 mV. For 10 mM Ca^{2+} (Fig. 3*B*), γ was equal to 13 pS and V_r was around +25 mV. Thus, one can consider with certainty that the GTPγS-induced channels recorded in outside-out patches are the same as the ATP-activated ones.

Dependence of Unitary Current Amplitude on External Ca^{2+} Concentration

Figure 3*A* shows typical current records from an outside-out patch with different Ca^{2+} concentrations in the bath solution. Figure 3*B* presents the current-voltage relations. Current amplitudes are the mean from 4 to 7 experiments for 2, 10 and 105 mM Ca^{2+} and from one experiment for 20 and 40 mM Ca^{2+} as the only current carrier. The mean values of unitary current at −30 mV are plotted as a function of Ca^{2+} concentration in Fig. 3*C*.

It can be seen that unitary current Ca^{2+} currents display a tendency to saturate with the increase of Ca^{2+} concentration. Fitted saturation curve gives concentration value for the apparent dissociated constant (K_d) of 10 mM for Ca^{2+}.

Channel Selectivity for Various Divalent and Monovalent Cations

Values of +53 mV and +25 mV for V_r could be obtained by extrapolating the current-voltage relationships at 105 and 10 mM Ca^{2+} shown in Fig. 3*B*. According to the V_r data, relative permeability for Ca^{2+} *vs.* K^+ could be estimated using following modification of the Goldman-Hodgkin-Katz equation (Hille, 1992):

$$V_r = (RT/2F)\, ln(4P_{Ca}A_{Ca}/P_KA_K)$$

where A_{Ca} and A_K are the Ca^{2+} and K^+ activities, respectively. Activity coefficients were taken as 0.75 for 145 mM K^+ and 0.25 and 0.3 for 105 mM and 10 mM Ca^{2+}, respectively. *R, T* and *F* have their usual meaning. The values of P_{Ca}/P_k calculated for two calcium concentrations were equal to 68 and 66 and very close to that obtained in cell-attached and outside-out recordings with 10 mM Ca^{2+} as current carrier for ATP-activated Ca^{2+}-permeable channels (P_{Ca}/P_K = 71) (Naumov et al., 1995*a*).

To characterize the relative permeability of ATP-activated channels to various divalent and monovalent cations, we carried out current recordings under conditions when isomolar concentrations of different divalent cations (105 mM) or Na^+ (145 mM) were applied to the external surface of the patch. Four or five cation species could be tested on the same patch. Figure 4 shows an example of current records obtained in one typical experiment. Figure 5 presents current amplitude values *vs.* membrane potential, collected from 6 to 8 experiments, for divalent cations. Judged by extrapolated V_r, the relative selectivity of the channel for divalent ions follows the sequence $Ca^{2+} > Ba^{2+} > Mn^{2+} > Sr^{2+}$. Respective values of V_r are equal to +53, +43, +41 and + 29 mV. According to a Goldman-Hodgkin-Katz equation, permeability ratios are $Ca^{2+} : Ba^{2+} : Mn^{2+} : Sr^{2+} : K^+ = 68 : 30 : 26 : 10 : 1$.

Measurements of unitary channel currents with various charge carriers reveal significantly different selectivity sequences. Slope conductances for permeant divalents (at 105 mM as the charge carrier) rank as follows: Ca^{2+} (19 pS) $\geqslant Sr^{2+}$ (18 pS) > Mn^{2+} (12 pS) > Ba^{2+} (10 pS). For Sr^{2+}, Mn^{2+} and Ba^{2+}, this is opposite to the sequence from V_r determinations.

To estimate P_{Na}/P_K, experiments with application of the Ca^{2+}-free solution containing 145 mM Na^+ to the external membrane surface were carried out. In Ca^{2+}-free solutions, the patch membrane was unstable in some experiments. Activity of calcium-impermeable sodium-selective channels (Negulyaev & Vedernikova, 1994) was observed in a number of patches making it difficult to measure systematically sodium currents through ATP-activated channels. In three favorable experiments, one of which is shown in Fig. 7 (*see also* Fig. 4), extrapolated reversal potential with 145 mM external Na^+ was found to be +31 mV that suggests that the channel selects for Na^+ over K^+. Single channel conductance was 14 pS for 145 mM Na^+ and the unitary sodium currents through the ATP-activated channel were much smaller than those for comparable concentration of Ca^{2+} with intermediate values between Sr^{2+} and Mn^{2+} currents. According to V_r determination, P_{Na}/P_K is equal to 3.5.

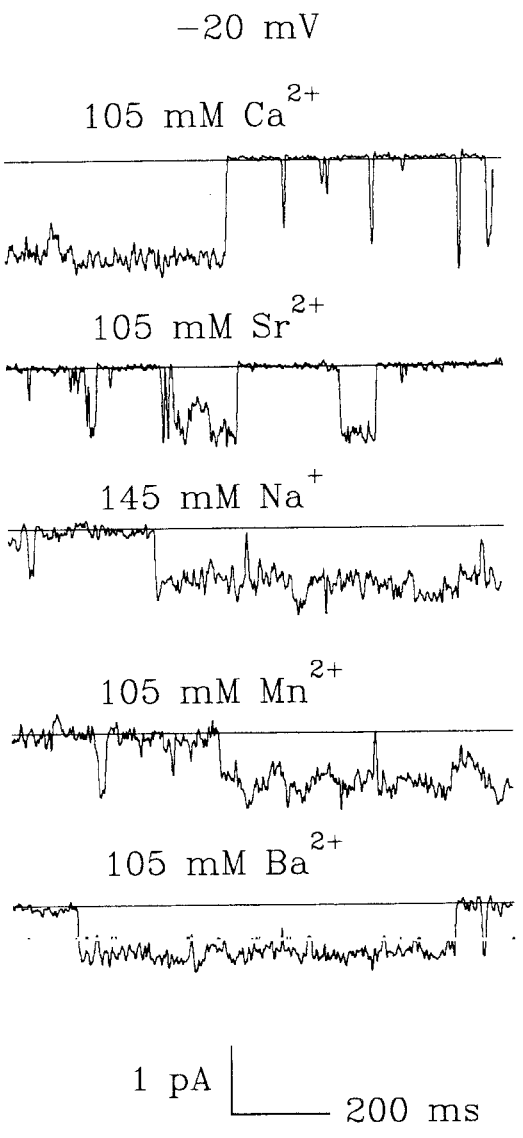

Fig. 4. Representative current records from the outside-out patch with the different cations in the external solution. Intracellular solution is the same as in Fig. 2. Holding potential was −20 mV.

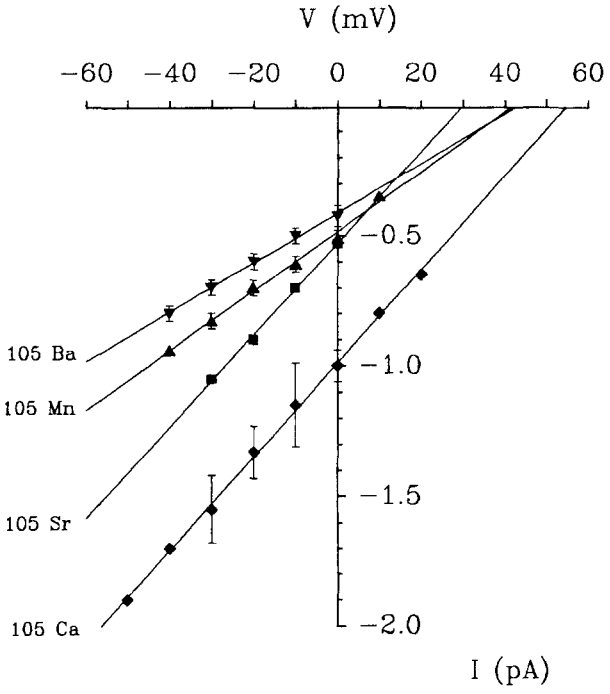

Fig. 5. Plot of the current amplitude values *vs.* membrane potential for various divalent cations in the external solution indicated with different symbols; concentrations are given in mM. Current amplitudes are the mean from 6 to 8 experiments. Error bars denote SEM.

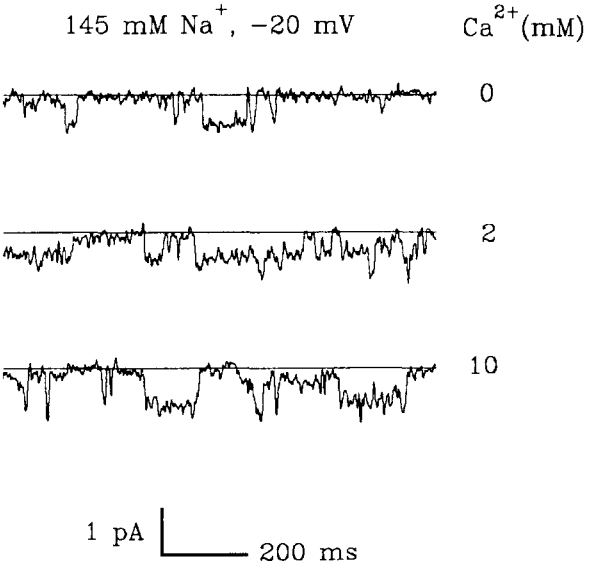

Fig. 6. Sodium currents through the Ca^{2+}-permeable channels at different external Ca^{2+} concentrations indicated in mM on the right. Holding potential was −20 mV. The same internal solution as in experiment presented in Fig. 2.

The inhibitory effect of micromolar concentrations of extracellular Ca^{2+} on Na^+ current was shown at the single channel level for voltage-gated Ca^{2+} channels (Kostyk, Mironov & Shuba, 1983; Almers, McClesky & Palade, 1984). For ATP-activated channels the inhibition of sodium current has been described in millimolar range of Ca^{2+} concentrations on smooth muscle (Benham & Tsien, 1987) and pheochromocytoma cells (Nakazawa et al., 1990*b;* Neuhaus et al., 1991). In both cases the sodium currents in Ca^{2+}-free solution were much larger than those registered in the isotonic Ca^{2+} solution. It is not true for our outside-out experiments on rat macrophages. It can be seen from Fig. 6 that addition of 2 mM and 10 mM Ca^{2+} to the external solution containing 145 mM Na^+ evoked no marked decrease of the current amplitude. One more experiment in which 2 mM Ca^{2+} were added to the external solution containing 145 mM Na^+ (Fig. 7) confirmed this observation; moreover, associated current-voltage relation (Fig. 7*B*) indicates slight increase of current amplitude.

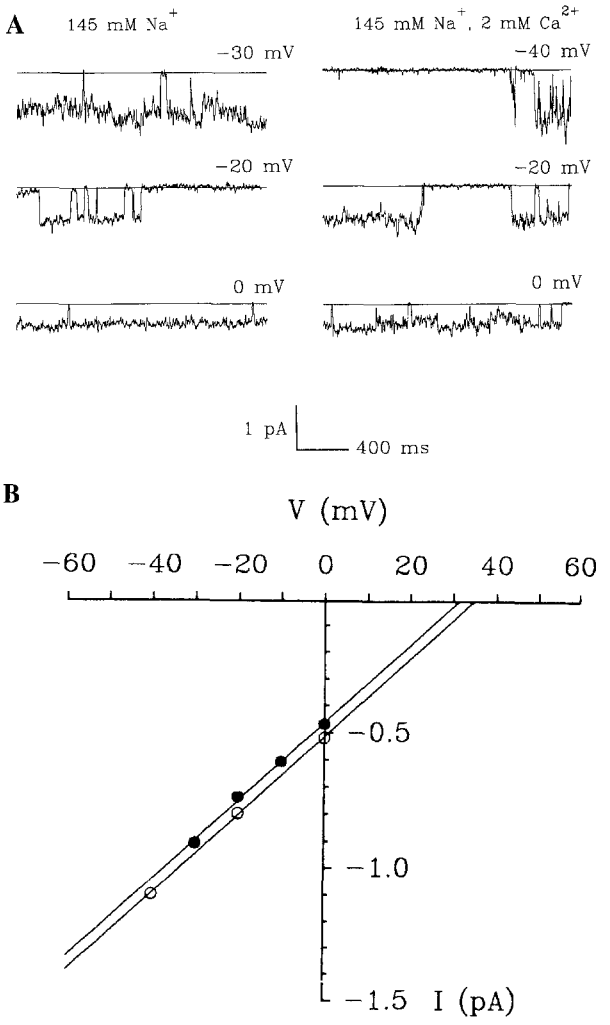

Fig. 7. Sodium currents through the Ca^{2+}-permeable channels in outside-out experiment. (*A*) Current traces at different holding potentials with 145 mM Na^+ in Ca^{2+}-free external solution (left) and with 145 mM Na^+ plus 2 mM Ca^{2+} (right) as the current carriers. Holding membrane potentials are indicated near the traces. (*B*) Associated current-voltage relations. Hollow symbols correspond to Ca^{2+}-free solution; filled ones—to solution contained 2 mM Ca^{2+}. Internal solution is the same as in the experiment shown in Fig. 2.

Discussion

One of the most important aspects of this work is that the channel activity in outside-out excised patches can be evoked in the presence of GTPγS in the pipette without any agonist applied to the external surface of the membrane. General characteristics of the channels (conductance and selectivity) are identical to those of ATP-activated Ca^{2+}-permeable channels described earlier (Naumov et al., 1995*a,b*). This fact strongly suggests that activation of some G protein is sufficient to gate the channels without the ATP receptor being occupied. The data are in discrepancy with the widely held concept of a direct coupling of ATP receptor with the channel (Benham & Tsien, 1987; McMillan et al., 1988; Neuhaus et al., 1991). In lacrimal acinar cells (Sasaki & Gallacher, 1992), GTP and GTPγS produced a marked potentiation of ATP-evoked response that suggests coupling of the ATP receptor with a small selective cationic channel via a G protein.

The results obtained in our current and previous studies suggest that the ATP receptor activation in rat macrophages results in the gating of a special channel with the properties different from those on other cell species. The ATP-activated channels have a rather high selectivity for divalent cations (especially for Ca^{2+}) *vs.* monovalent ones. The permeability ratios estimated from extrapolated V_r measurements are $P_{Ca} : P_{Ba} : P_{Mn} : P_{Sr} : P_{Na} : P_K = 68 : 30 : 26 : 10 : 3.5 : 1$. It should be noted that the reversal potential values may be underestimated as no outward currents were detected up to +50 mV in our experiments. This fact indicates possible nonlinearity of the current-voltage relations in this region. As a result, the relative permeability values for divalent cations also may be underestimated. The data available on selective properties of ATP-activated channels on other cell types are based mainly on whole-cell recordings (Benham & Tsien, 1987; Nakazawa & Matsuki, 1987; Sasaki & Gallacher, 1990; Vincent, 1992) of macroscopic ATP-induced currents performed with extracellular solutions containing Na^+ as the main cation (with or without addition of 1.8–2 mM Ca^{2+}) and with Cs^+ or K^+ as the main cation in the recording pipette. In these ionic conditions the value of E_r was found to be very close to 0 mV. The conclusion was made that an ATP-activated channel might be classified as a low selective cation channel permeable for monovalent cations and calcium. In the whole-cell experiments on smooth muscle (Benham & Tsien, 1987) the selectivity of the ATP-activated channels was evaluated by measuring of E_r in various external and internal solutions. The permeability ration P_{Ca}/P_K was obtained to be equal to 3.3 and permeability rations $P_{divalent}/P_{Cs}$ ranked $P_{Ca} : P_{Ba} : P_{Cs} = 2.7 : 1.9 : 1$. In the endothelial cell membrane (Yamamoto et al., 1992), a histamine-activated cation channel was described as selective to Ca^{2+} but also permeable to Na^+ and K^+ with the permeability ratios $P_{Ca} : P_{Na} : P_K = 15.7 : 1 : 1$. The ryanodine receptor channel displays permeability differences between K^+ and the divalent cations with $P_{divalent}/P_K$ of about 6 and with little difference in permeability among the individual divalents (Tinker & Williams, 1992). P_{Ba}/P_K of 6.3 was estimated for inositol (1,4,5)-trisphosphate-gated Ca channels from cerebellum (Bezprozvanny & Ehrlich, 1994). Thus, ATP-activated channels described in our study seem to be related to a rather high selective Ca^{2+} channel type. They, however, are much less selective as compared to the voltage-gated channels, in particular to the L-type channels with $P_{Ca} : P_K > 1000$ (Hess, Lansman & Tsien, 1986). Measurements of unitary channel currents with various charge carriers reveal quite different selectivity sequences. Slope conductances for permeant divalents at 105 mM rank as follows: $Ca^{2+} \geqslant Sr^{2+} > Mn^{2+} > Ba^{2+}$. For Sr^{2+}, Mn^{2+} and Ba^{2+} this is essentially opposite to the sequence from V_r determinations. Interpretation of the results requires suggestion of a mechanism of selectivity involving different ion affinities as a binding site, corresponding to an energy well (Hess & Tsien, 1984; Almers & McClesky, 1984; Tinker, Lindsay & Williams, 1992). Measurements of ionic current as a function of Ca^{2+} concentration provide for information about binding of Ca^{2+} to saturable sites within the pore. The concentration value for the apparent K_d of 10 mM is similar to that found for L-type Ca^{2+} channels (Hess et al., 1986). However, the permeability sequence determined by γ differs significantly from that obtained for L-channels because Ba^{2+} was found to be an ion with the lowest single channel conductance among all divalents tested in our work. Na^+ current measurements also revealed a difference. Both for L-channels, and for ATP-activated channels described on some types of cells (Benham & Tsien, 1987; Sasaki & Gallacher, 1990), the sodium single channel conductance is higher than the calcium one. On rat macrophages, however, it is lower than γ for Ca^{2+} (14 pS, respectively), with intermediate value between γ for Mn^{2+} and Ba^{2+}.

We found no indication of the block by external Ca^{2+} of Na^+ permeation in millimolar range of Ca^{2+} concentrations which have been described for ATP-activated channels on pheochromocytoma cells (Nakazawa & Hess, 1993; Neuhaus et al., 1991). These results give additional evidence for the ATP-activated GTP-dependent channels on rat macrophages have to be considered a peculiar channel type.

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