Original Article
Electrophysiology properties of voltage-gated potassium channels in rat peritoneal macrophages

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Abstract: Ion channels are important for the functions of excitable and non-excitable cells. Using the whole-cell patch clamp technique, we analyzed the electrophysiological and pharmacological properties of voltage-gated potassium channels in primary rat peritoneal macrophages. With intracellular solution contained K+ as the main charge carrier, all cells showed outward currents in response to membrane depolarization. The currents can be inhibited by TEA (10 mM), a non-selective blocker for voltage-gated K+ channels, and attenuated when intracellular K+ was substituted with Cs+. Changing holding potential from -80 to -30 mV or -10 mV also inhibited the outward currents. In contrast, increasing the concentration of ATP in the intracellular solution decreased the amplitude of the outward currents. Thus, rat peritoneal macrophages express several types of functional voltage-gated K+ channels.

Keywords: Patch-clamp, peritoneal macrophage, potassium channel, TEA, ATP

Introduction
Macrophages are professional antigen-processing cells. They can trigger cytokine secretion, stimulate T cell signaling, and play a pivotal role in the initiation of the inflammatory response to injury or infection. Ion channels have been shown to be important for the activation of macrophages [1]. For example, changes in membrane potentials are among the earliest detectable events upon stimulation of phagocytosis [2]. Previous studies have shown that bone marrow-derived macrophages (BMDM) express voltage-gated K+ channels and that association of Kv1.5 and Kv1.3 contributes to the majority of K+ channels in these cells [3, 4]. However, at present, no detailed characterizations of voltage-gated K+ channels in primary macrophages have been reported. Thus, the current study focuses on the electrophysiological and pharmacological characterization of voltage-gated K+ channels in primary rat peritoneal macrophages. We show that several types of functional K+ channels are expressed in these cells. Our results may lay the foundation for further studies of the functions and modulations of these channels in normal and pathological immune responses.

Materials and methods
Rat peritoneal macrophages extraction and culture
Rat peritoneal macrophages were isolated according to the previously described method [5]. The protocol of using rats for this study was approved by Institutional Animal Care and Use Committee of Anhui Medical University. 2-3 month old rats were anesthetized with ether followed by cervical dislocation. The rats were placed supine on the table and then soaking in benzalkonium bromide solution (1:50) for 3-5 minutes. The skin of the abdominal region was cut and the peritoneal cavity was lavaged with cold PBS (10 ml) for 2-3 minutes. After 3 minutes, the peritoneal fluid was collected using a transfer pipette. The peritoneal cells were isolated with centrifugation and suspension for several times. The cells were plated into 35 mm
diameter culture dishes contained 3 ml DMEM. The medium was changed after 3 h to wash out the cells which had not adhered. The cells were incubated with DMEM for 12-24 h before recording. Using the same method, a previous study has shown that >80% cells were macrophages [5].

Electrophysiological recordings

Whole-cell currents in peritoneal macrophages were recorded using the patch-clamp technique [6]. The currents were recorded using the MultiClamp 700B amplifier (Molecular Device, USA), low-pass filtered at 2 KHz, digitized using the Digidata 1440A analogue-to-digital converter. Recording electrodes were made from glass micropipettes (0.86 mm diameter, Sutter Instrument) using a multi-stage micropipette puller (P-97, Sutter, USA). The range of resistance was 3-5 MΩ, when filled with the intracellular solutions. After a tight GΩ seal was formed, the patch membrane was ruptured by applying strong suction along with a few zapping pulses with duration ranging from 1 to 5 ms. Currents were recorded 3 minutes after the formations of whole-cell configuration to allow for the equilibration between the cell and pipette solution. Unless otherwise stated, holding potential for most cells was set at -60 mV. Membrane currents were recorded with test potentials between -80 and 100 mV with a 10 mV increment. As shown in Figure 1A, substantial outward currents were activated at membrane potentials positive to -50 mV. The amplitude of the outward currents, measured at the end of 250 ms voltage pulses, increased with membrane depolarization and reached plateau at ~0 mV. Positive to 0 mV, however, increasing membrane depolarization produced less outward current (Figure 1A). Fitting the currents recorded

Statistical analysis

Data are shown as mean ± standard error of the mean (s.e.). Paired or unpaired Student’s t-test was used for the analysis of statistical difference between mean values. A p value of <0.05 was considered significant.

Results

Membrane currents recorded in rat peritoneal macrophages

Following the formation of gigaohm seal and whole-cell configuration, the cell capacitance ($C_m$) and series or access resistance ($R_s$) were recorded. In 30 cells recorded, $C_m$ was 7.5 ± 0.5 pF and $R_s$ was 10.5 ± 4.5 MΩ without compensation. 3 minutes period was allowed for equilibration between cell interior and pipette solution before recording the current. Unless otherwise stated, holding potential for most cells was set at -60 mV. Membrane currents were recorded with test potentials between -80 and 100 mV with a 10 mV increment. As shown in Figure 1A, substantial outward currents were activated at membrane potentials positive to -50 mV. The amplitude of the outward currents, measured at the end of 250 ms voltage pulses, increased with membrane depolarization and reached plateau at ~0 mV. Positive to 0 mV, however, increasing membrane depolarization produced less outward current (Figure 1A). Fitting the currents recorded
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between -80 to 100 mV with Boltzmann function, yielded a half maximal activation potential $V_{1/2} = -25.69 \pm 2.23$ mV and a slope factor $k = 2.73 \pm 0.49$ mV (Figure 1B, $n = 9$).

**Effect of TEA on the outward currents in rat peritoneal macrophages**

Next, we tested the effect of TEA (10 mM), a commonly used K+ channel blocker [7], on the outward currents in rat peritoneal macrophages. As shown in Figure 2, perfusion of cells with TEA for ~3 min effectively suppressed the outward currents. The peak amplitude of the outward currents, recorded at 0 mV, was suppressed from 264.05 ± 15.30 pA to 70.23 ± 8.41 pA ($p<0.05$, $n=6$). The blockade of TEA on the outward currents was reversible. After washing out of TEA from the extracellular solution, the peak amplitude of the outward currents at 0 mV was 224.52 ± 8.41 pA (Figure 2A and 2B). This finding strongly suggests that the outward currents in these cells were carried by voltage-gated K+ channels. Consistent with the K+ channels being activated, the currents almost disappeared when intracellular K+ was substituted with Cs+, a non-permeate ion for K+ channels (Figure 3). With Cs+ substituting K+ in the intracellular solution, the peak amplitude of the outward currents decreased from 312.28 ± 19.80 pA to 78.92 ± 5.84 pA ($p<0.01$, $n=6$).

**Influence of changing holding potential on the outward currents in rat peritoneal macrophages**

Previous studies have shown that different components of K+ currents have different sensitivity to changing holding potentials. For example, the delayed rectifier K+ currents can

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**Figure 2.** Effect of external TEA on the outward currents recorded in rat peritoneal macrophages. A. Example traces showing the outward currents before and after 10 mM TEA. B. Summary data showing average I-V curve for outward currents in the absence and presence of 10 mM TEA, $n = 6$. 
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be dramatically suppressed by changing the holding potential to slightly depolarized voltage (e.g., -30 mV) [8]. To further characterize the outward currents in rat peritoneal macrophages, we study the effect of changing holding potential on the amplitude of outward currents. As shown in Figure 4, changing the holding potential from -80 to -30 or -10 mV dramatically decreased the amplitude of the outward currents (Figure 4). Mean peak amplitudes of the outward currents, recorded at the test potential of 0 mV, for example, were 254.35 ± 46.25 pA, 91.59 ± 28.62 pA, and 22.05 ± 7.89 pA with a holding potential of -80, -30, and -10 mV, respectively (n=6).

Influence of MgATP on the outward currents in rat peritoneal macrophages

ATP-sensitive potassium (K<sub>ATP</sub>) channels serve as a metabolic sensor, coupling cell metabolism to plasmalemmal potassium fluxes and
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Figure 4. The outward currents in rat peritoneal macrophages were inactivated by depolarized holding potentials. A. Example traces showing the outward currents recorded with a holding potential of -80 mV, -30 mV, or -10 mV. B. Averaged I–V curves recorded with a holding potential of -80 mV, -30 mV, or -10 mV (n = 6).

Electrical activity in different cell types. Their activity is primarily determined by intracellular adenosine nucleotides, with ATP having an inhibitory effect on the channel activity [9]. With an increased concentration of MgATP in the pipette solution from 0 mM to 4 mM, the outward currents at test potentials positive than +20 mV decreased significantly (Figure 5). For example, at +60 mV, the amplitudes of the outward currents were 525.50 ± 81.97 pA and 191.504 ± 8.24 pA with intracellular solutions contained 0 mM and 4 mM MgATP (n=5 and 6, p<0.05). Interestingly, the lack of ATP caused a slight decrease in the amplitude of the outward currents at test potentials negative than +10 mV (Figure 5B). The reason for this shift is unclear. It is likely that changes in protein phosphorylation might be involved.

Discussion

Macrophages play critical functions in the immune system, acting as regulators in homeostasis and as effector cells in infection, injury, and tumor growth. In response to different growth factors and cytokines, macrophages can proliferate, become differentiated or activated [4]. Voltage-dependent potassium channels (Kv) play a vital role in normal cell functions by determining the resting membrane potential in both excitable and non-excitable cells and controlling action potential firing in excitable cells [10]. More and more evidence indicates that Kv channels are involved in immunoregulation. For example, Kv1.3 channels expressed in T cells determine the state of lymphocyte activation and differentiation [11].
The detailed electrophysiological and pharmacological properties and potential functions of Kv channels in peritoneal macrophages are, however, less reported.

In this study, whole-cell currents in rat peritoneal macrophages were measured using the patch-clamp technique. Two major components of the outward currents can be seen in these cells: a slow and non-desensitizing component and a fast transient component. These two major current components have some characteristics of delayed rectifier K+ currents and A-type K+ currents commonly seen in other cell types, e.g. mammalian neurons [7]. TEA is a common K+ channel blocker which blocks most of Kv channels [7]. In rat peritoneal macrophages, the outward currents were dramatically suppressed by 10 mM TEA, confirming the presence of functional Kv channels in these cells.

Figure 5. Increasing the concentration of MgATP in the intracellular solution inhibits the outward currents in rat peritoneal macrophages at test potentials positive than +20 mV. A. Example traces showing outward currents recorded with 0 mM and 4 mM MgATP in the pipette solution. B. Averaged I-V curves obtained with intracellular solutions contained either 0 mM (n = 6) or 4 mM MgATP (n = 5).
cells. Consistent with $K^+$ channels being activated, the outward currents were eliminated when intracellular $K^+$ was substituted with Cs$^+$. Ion channels open and close in response to different factors, such as neurotransmitter, membrane tension, temperature and membrane potential. Voltage-gated ion channels are extremely sensitive to changes in membrane potential. The open probability for voltage-gated Na$^+$ or K$^+$ channels, for example, may increase as much as 10 times with every 5 mV depolarization [12, 13]. In addition to channel activation, changing holding potential can produce inactivation of some channels which dramatically alters the availability of those channels to be activated. For example, voltage-gated Na$^+$ channels can be largely inactivated at a slightly depolarized holding potential. Due to the difference in the sensitivity to voltage-dependent inactivation, changing holding potential has also been used to separate different components of voltage-gated K$^+$ currents. For example, in vascular smooth muscle cells, changing holding potential to -30 mV or higher can completely inactivate the delayed rectifier K$^+$ channels without any effect on large conductance Ca$^{2+}$-activated K$^+$ channels [14]. Our studies show that the K$^+$ currents in rat peritoneal macrophages can also be inhibited by using depolarized holding potentials. At a holding potential of -30 mV, $K_{DR}$-like currents almost completely disappeared while $K_{CA}$-like current existed. At a holding potential of -10 mV, however, both currents were dramatically inactivated.

ATP-sensitive potassium ($K_{ATP}$) channel couple cell metabolism to cell excitability. They play a variety of physiological roles in many tissues. For example, they are of key importance in pancreatic β cells, where they couple glucose metabolism to insulin secretion [15, 16]. The magnitude of the $K_{ATP}$ current dictates the degree of insulin release. The archetypal $K_{ATP}$ channel is an octameric complex of kir6.2 and SUR subunits [17]. The hallmark of $K_{ATP}$ is the sensitivity to inhibition by intracellular ATP. This is consistent with the results that the current amplitudes decreased when concentration of MgATP was increased from 0 to 4 mM. Interestingly, the amplitudes of the outward currents at test potentials negative than +10 mV were potentiated by the presence of MgATP. Since phosphorylations of ion channels by protein kinases regulate the activities of various ion channels including voltage-gated K$^+$ channels [18], it is possible that the increase in the amplitude of the outward current at moderately depolarized membrane potentials in the presence of MgATP is due to an increased phosphorylation of some type of voltage-gated K$^+$ channels. Future studies using protein kinase inhibitors may provide useful information.

In summary, our current studies suggest that primary rat peritoneal macrophages express different types of functional voltage-gated K$^+$ channels.

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