Membrane-delimited Regulation of Novel Background K⁺ Channels by MgATP in Murine Immature B Cells

Joo Hyun Nam, Ji-Eun Woo, Dae-Yong Uhm, and Sung Joon Kim†‡

From the Department of Physiology, Center for Molecular Medicine, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, Korea

In WEHI-231, a representative immature B cell line, Ca²⁺ entry is paradoxically augmented by treatment with 2-aminoethoxydiphenyl borate (2-APB), a blocker of inositol 1,4,5-trisphosphate receptor and of nonselective cation channels (Nam, J. H., Yun, S. S., Kim, T. J., Uhm, D.-Y., and Kim, S. J. (2003) FEBS Lett. 535, 113-118). The initial goal of the present study was to elucidate the effects of 2-APB on membrane currents, which revealed the presence of novel K⁺ channels in WEHI-231 cells. Under whole-cell patch clamp conditions, 2-APB induced background K⁺ current (Ibg) and hyperpolarization in WEHI-231 cells. Lowering of intracellular MgATP also induced the Ibg. The Ibg was blocked by micromolar concentrations of quinidine but not by tetraethylammonium. In a single channel study, two types of voltage-dependent K⁺ channels were found, with large (346 picosiemens) and medium conductance (112 picosiemens) channels, named BKbg and MKbg, respectively. The excision of membrane patches (inside-out (i-o) patches) greatly increased the Pₜ of BKbg. In i-o patches, cytoplasmic MgATP (IC₅₀ = 0.18 mM) decreased the BKbg activity, although non-hydrolyzable adenosine 5'-[(βγimino)triphosphate]) that is mainly determined by the potassium permeable cell membrane and transmembrane gradient of [K⁺]. K⁺ channels, besides setting resting Vm, play critical roles in various cellular functions (1). Among them, the modulation of calcium signals by providing electrical driving force has been suggested as an essential role of K⁺ channels (1, 2). In human primary T cells, two kinds of K⁺ channels, the voltage-gated K⁺ channels Kv1.3 and the calcium-activated K⁺ channel IKCa1 (hSK4), are found to play such a role; Kv1.3 channels are essential for activation of quiescent cells, and signaling through protein kinase C pathway enhances expression of IKCa1 channels that are required for proliferation (3, 4). Besides Vm regulation, K⁺ channels also regulate the loss of intracellular K⁺, which is a prerequisite step in the normotonic- or the Fas-mediated apoptosis (5, 6). In contrast to the studies in T cells, the characteristics of K⁺ channels and their roles in B cells have been rarely investigated, and the types of reported K⁺ channels are restricted to the voltage-dependent (Kv) and Ca²⁺-activated (KCn) channels (7, 8).

A distinctive feature of the immune system is the balanced fine-tuning between growth and death by apoptosis. In bone marrow, the immature B cells with membrane-bound immunoglobulins reactive to autoantigens are arrested in the cell cycle and eliminated through the process of apoptosis, a crucial step preventing autoimmune diseases. In contrast, the mature B cells, once activated by specific antigens, undergo a second round of proliferation and selection in the secondary lymphoid organs to differentiate into memory B cells (9, 10). WEHI-231 cells are the representative murine B lymphoma cell line that reflects the characteristics of immature B cells, apoptosis by cross-linking B cell receptors (BCR) (11-13). The BCR ligation activates a series of protein-tyrosine kinases. Several of them (e.g. Syk and Btk) are involved in the activation of phospholipase Cγ, producing IP₃ that subsequently mobilizes intracellular Ca²⁺ stores via the IP₃ receptor (9, 10, 14). The resulting decrease in the Ca²⁺ content of intracellular stores triggers store-operated Ca²⁺ entry (SOCE), which is required for the tonic increase in cytosolic Ca²⁺ concentration ([Ca²⁺]). (8, 15).

In our previous study, the increase in [Ca²⁺], by SOCE was compared between cell lines Bal 17 (mature B cells) and WEHI-231 (16). In WEHI-231 cells, the increase in [Ca²⁺] is paradoxically augmented by an application of 2-aminoethoxydiphenylborate (2-APB) that has been reported to block a SOCE channel, and the unexpected effects might imply a 2-APB-activated Ca²⁺-permeable channels in WEHI-231, as
recently identified in basophilic leukemia cells (18). Another possibility is that 2-APB indirectly enhances the Ca\(^{2+}\) influx via increasing the electrical driving force (i.e., membrane hyperpolarization). In the latter case, the K\(^{+}\) conductance of the WEHI-231 cell membrane would be increased by 2-APB. To address such hypotheses, it is essential to measure directly the membrane currents of WEHI-231 cells and examine the effects of 2-APB.

Therefore, in this study, the initial goal was to elucidate the membrane conductance of WEHI-231 cells that is positively regulated by 2-APB. To our surprise, the patch clamp study indicates the presence of novel background K\(^{+}\) channels with very large unitary conductance (>340 pS) that are positively regulated by 2-APB. In addition, background K\(^{+}\) channels with medium size conductance (112 pS) are also present in WEHI-231 cells. Our experimental results also demonstrate that the large conductance background K\(^{+}\) channels are negatively regulated by cytoplasmic ATP via phosphorylation of phosphoinositides in a membrane-delimited manner.

**EXPERIMENTAL PROCEDURES**

**Cells**—Mouse B lymphocytes with properties of immature B cells (WEHI-231) and mature B cells (Balb/17) were grown in 25 cm \(^2\) HEPEX RPMI 1640 media (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HyClone, Logan, UT), 50 \(\mu\)g 2-mercaptoethanol (Sigma), and 1% penicillin/streptomycin (Invitrogen). All cells were incubated at 37 \(^\circ\)C in 5% CO\(_2\). The bath (5 ml) was superfused at 5 ml/min, and voltage clamp experiments were performed at room temperature (22 ± 2 \(^\circ\)C). Patch pipettes with a free-tip resistance of about 2.5 megohms were connected to the head stage of a patch-clamp amplifier (Axopatch-1D, Axon Instruments). Liquid junction potentials were corrected with an offset circuit before each experiment. Unless mentioned otherwise, a conventional whole-cell clamp was achieved by rupturing the patch membrane after making a giga-seal. In the perforated whole-cell patch clamp, a stock solution of nystatin in dimethyl sulfoxide (15 mg/ml) was added to the pipette solution, yielding a final concentration of 0.15 mg/ml. A steady-state perforation was usually achieved within 5 min after making a giga-seal. pCLAMP software version 7.0 and Digidata-1200A (both from Axon Instrument) were used for the acquisition of data and the application of command pulses. The resting membrane potential described in this study was measured under the zero-current clamp condition of the whole-cell patch clamp. Voltage and current data were low pass filtered (5 kHz) and stored using a digital tape recorder (DTR-1205, Biological, Claix, France). Current traces were stored in a Pentium-grade computer and analyzed using pCLAMP software version 6.0 and Origin version 6.1 (Microsoft Software Inc., Northampton, MA). Single channel activities were recorded at 10 kHz in cell-attached (c-a) and inside-out (i-o) configurations using fire-polished glass pipettes (final resistance, 8–9 megohms). Recordings were performed at room temperature with Axopatch-200B (Axon Instruments). The voltage and current data were low pass filtered at 2 kHz and stored for later analysis using Fetchan and pSTAT version 6.0 software (Axon Instruments). Data were analyzed to obtain an amplitude histogram and open probability \((P_0)\). Data were represented as mean ± S.E. Student’s \(t\) test was used to test for significant differences among the means. For reasons yet unknown, the amplitude of \(I_{K,bg}\) tended to decrease with the lapse of culture date. Therefore, throughout the present study, care was taken to compare the effects of experimental conditions with the responses of control cells on the same date of culture. In the next experiments, the conventional whole-cell clamp was applied to control the intracellular environment more precisely (i.e., excluding the possibility of Ca\(^{2+}\) increase). A striking feature of WEHI-231 cells was that an outward current increased spontaneously after breaking in the patch membrane and dialyzing with MgATP-free KCl solution. On making a whole-cell configuration, the initial resting membrane potential was fluctuating around ~30 mV, which hyperpolarized spontaneously to ~75.0 ± 1.56 mV within 5–6 min after the break-in (\(n = 13\)). During the experiment, the membrane voltage was intermittently clamped at ~60 mV, and ramp pulses (Fig. 1, C and D) were applied to monitor the changes in the membrane conductance. The slope of spontaneously developed...
membrane conductance was basically voltage-independent (Fig. 1C, \( n = 5 \)), same with the \( I_{\text{k,bg}} \) activated by 2-APB (Fig. 1, A and B). After full activation of \( I_{\text{k,bg}} \) by the dialysis with the MgATP-free pipette solution, 2-APB had no further effect (\( n = 2 \), data not shown). The intracellular dialysis with di-sodium ATP (Na\(_2\)ATP, 3 mM) without adding Mg\(^{2+}\) similarly increased the amplitude of \( I_{\text{k,bg}} \) (Fig. 1C, \( n = 5 \)). In contrast, no development of outward current was observed when 3 mM MgATP was

**Fig. 1. Background-type K\(^+\) currents in WEHI-231 cells.** A, membrane currents in control (left panel) and 2 min after 2-APB application (50 \( \mu \)M, right column) obtained by step pulses (see inset) under the nystatin-perforated whole-cell clamp conditions. B, I/V curves obtained by depolarizing ramp pulses from \(-90\) to \(60\) mV in control and 2 min after 2-APB application (mean \( \pm \) S.E., \( n = 6 \)). C, summary of steady-state I/V curves after dialyzing the cells with KCl pipette solutions containing no MgATP, 3 mM Na\(_2\)ATP, and with 3 mM MgATP (mean \( \pm \) S.E., \( n = 5 \)), respectively. Note that the initial I/V curve just after breaking-in patch membrane (control) is overlapped with the response to 3 mM MgATP. D, with 3 mM MgATP in pipette solution, 2-APB (50 \( \mu \)M) induced a small increase in outward current and the leftward shift of I/V curves (\( n = 3 \)). E, after confirming the steady-state outward current under MgATP-free conditions, the extracellular concentration of KCl was increased by replacing with equimolar NaCl as indicated in the figure. F, no development of background-type K\(^+\) current in Bal-17 cells dialyzed with MgATP-free KCl solution (\( n = 5 \)). The I/V curves obtained in the initial control (filled squares), 5 min after break-in (filled circles), and addition of 2-APB (50 \( \mu \)M, open triangles).
In WEHI-231 cells, after dialyzing with MgATP-free pipette solution over several minutes to develop IKbg, a bath application of 10 mM TEA weakly decreased the outward currents only at depolarized clamp voltages (>40 mV) without shifting the reversal potential of the I/V curves (Fig. 1F). In this state, the application of 2-APB did not evoke IKbg but reduced the currents at depolarized clamp voltages (Fig. 1F).

The IKbg Is Resistant to TEA but Sensitive to Quinine/Quinidine—Tetraethylammonium (TEA) is an ion channel blocker with broad effects on various classes of K+ channels. In WEHI-231 cells, after dialyzing with MgATP-free KCl solution, and various levels of depolarizing step pulses were applied. A prolonged dialysis with MgATP-free solution (>5 min) induced an increase of outward current only at highly depolarized clamp voltages (>40 mV) without shifting the reversal potential of the I/V curves (Fig. 1F). In this state, the application of 2-APB did not evoke IKbg but reduced the currents at depolarized clamp voltages (Fig. 1F).

In the next experiment, therefore, the effects of quinine and quinidine on the IKbg were examined. After confirming the full activation of IKbg and membrane hyperpolarization under MgATP-free conditions, all extracellular Na+ was substituted to K+ (140 mM KCl), which revealed inward K+ currents at negative membrane voltages. The brief I/V curves were obtained with ramp-pulse protocols (from −90 to +60 mV, 0.1 V/s, 5-s interval), and various concentrations of blockers were applied to the bath (Fig. 2B). Both quinine and quinidine displayed inhibitory effects on IKbg in a completely reversible manner. The effects of quinine and quinidine were voltage-dependent; both agents blocked IKbg more effectively at positive voltages than at negative voltages. The concentration-response curves were obtained at −60 and at +60 mV, where the half-inhibitory concentrations (IC50) of quinine were 30.5 and 9.8 μM, respectively. Similarly, the IC50 values of quinidine were 51.7 and 6.2 μM at −60 and at +60 mV, respectively (Fig. 2C and D). We also tested the effects of Ba2+, a nonselective K+ channel blocker, on IKbg. The blocking effects of Ba2+ were relatively weak and more effective at negative membrane voltages, opposite to the effects of quinine or quinidine (Fig. 2E and F).

As some types of K2P channels are selectively regulated by extracellular pH and the stretch of cell membranes (19, 20), it was tested whether the extracellular acidification or osmotic stress affects IKbg. An acidification of bath solution to pH 6.5 decreased the peak amplitude of outward currents by 21 ± 1.8, 45 ± 9.6, and 54 ± 9.6% of control at +60, 0, and −40 mV, respectively (data not shown). Thus the inhibitory effect by extracellular acidification was weakly voltage-dependent; a larger inhibition was observed at the negative clamp voltage. In another experiment, to exert an osmotic stress, 30 mM extracellular NaCl was replaced with 60 mM sucrose and regarded as an isotonic control. To apply hypertonic and hypotonic stimuli, 60 mM sucrose was omitted (−60 mosm) or newly added (+60 mosm, total 120 mM sucrose) for 3 min, respectively. Although not directly shown here, concomitant swelling or shrinkage of cells was clearly observable. However, neither the hypertonic shrinkage nor the hypotonic swelling had significant effect on IKbg. Also, when the development of IKbg was suppressed with 3 mM MgATP in the pipette solution, the hypotonic swelling could not induce IKbg (data not shown).

Single Channel Recording of the Background K+ Channel—In the c-a condition with KCl (140 mM) pipette solution, single channel activities with large amplitudes of unitary currents were observed in WEHI-231 cells (Fig. 3A-a). The P0 was initially very low but was greatly increased by the excision of membrane into MgATP-free KCl solution (Fig. 3A-b, see also Fig. 3C). When the K+ in the cytoplasmic side was totally replaced with Na+, the outward current channel at the positive clamp voltage was abolished (Fig. 3A-c), which confirmed the K+ selectivity of this channel. In the i-o conditions, the I/V relation under symmetrical KCl showed a weak inward rectification (Fig. 3B, closed circles). The slope conductance was 346 pS at negative voltages, and the P0 was similarly high at both positive and negative membrane voltages (Fig. 3C). In the c-a recording with KCl pipette solution, presumably symmetrical K+ gradient across the patch membrane, the I/V curve was same with the one obtained under the i-o conditions (data not shown). In some cases of c-a recording, the KCl concentration in the pipette solution was reduced to 8.5 mM by an isomolar replacement with NaCl. With the low K+ pipette solution (i.e. low [K+]o), the reversal potential of I/V curve was −70 mV, again indicating the K+-selective permeability of the 346 pS K+ channel (Fig. 3B, open circles, n = 4).

From these results, we designated the background K+ channel with a maximum slope conductance of 346 pS as BKbg. The BKbg were observed in 95 cases out of total 480 trials of i-o patches. Although the recording of BKbg was done in Ca2+-free conditions with 1 mM EGTA, the size of unitary conductance, as well as the slope conductance were similar to those obtained under the i-o conditions (data not shown). In these, it was shown that the conductance and selectivity of this channel was 346 pS at negative voltages, and the P0 was similarly high at both positive and negative membrane voltages (Fig. 3C). In the c-a recording with KCl pipette solution, presumably symmetrical K+ gradient across the patch membrane, the I/V curve was same with the one obtained under the i-o conditions (data not shown). In some cases of c-a recording, the KCl concentration in the pipette solution was reduced to 8.5 mM by an isomolar replacement with NaCl. With the low K+ pipette solution (i.e. low [K+]o), the reversal potential of I/V curve was −70 mV, again indicating the K+-selective permeability of the 346 pS K+ channel (Fig. 3B, open circles, n = 4).

In addition to BKbg, K+ channels with smaller conductance, designated MKbg (medium conductance background K+ channels), were observed at both positive and negative clamp voltages (Fig. 4). In the i-o conditions, the I-V curve of MKbg also showed a weak inward rectification with maximum unitary conductance of 112 pS at negative membrane voltages (Fig. 4A). The replacement of cytoplasmic K+ with Na+ completely abolished the channel activity, proving the K+ selectivity of MKbg (Fig. 4B). Although the unitary conductance was larger
at negative voltages, the $P_o$ of MKbg was 2-fold higher at +60 mV than at -60 mV (Fig. 4C). The MKbg$^+$s were observed in 46 cases out of a total 480 trials of i-o patches. Because the unitary conductance of MKbg was similar to that of TREK-2 channels (22), it was tested whether the acidification of intracellular pH or membrane stretch facilitates the activity of MKbg. However, the intracellular acidification (pH 6.0) inhibited MKbg$^+$ ($n = 4$, Fig. 4D), and the membrane stretch by a negative pressure (-40 cm of H$_2$O in the pipette) had no effect ($n = 3$, Fig. 4E).

**Inhibition of BKbg by Intracellular MgATP**—As mentioned above, the excision of membrane patches yielded a large increase of the $P_o$ of BKbg$^+$ suggesting the washout of “inhibitory” cytosolic components. Because the whole-cell current ($I_{K,bg}$) was sensitive to MgATP in the cytoplasmic solution, we tested...
the effects of MgATP on the K⁺ channels under the i-o conditions. The $P_o$ of BKbg decreased dramatically with the application of MgATP (1 mM) to the cytoplasmic side, which was completely reversed by washout (Fig. 5A). In contrast, the di-sodium form of ATP (Na₂ATP) had no effect on BKbg activity (n/3, data not shown). BK bg activity was not affected by AMP-PNP (1 mM), a non-hydrolyzable analogue of ATP (Fig. 5B). These results suggest a phosphorylation-dependent regulatory mechanism for the inhibition of BKbg. Moreover, the application of ATP⁺S (1 mM), which is commonly used for permanent phosphorylation of substrates, exerted a non-washable inhibition of BKbg (Fig. 5C). Because the commercially available AMP-PNP and ATP⁺S are provided as lithium salts, 0.5 mM MgCl₂ was added along with the application of AMP-PNP or ATP⁺S in the above experiments. The application of Li₂ATP⁺S (1 mM) had no effect on BKbg activity (n = 2, data not shown). The current trace of Fig. 5C demonstrates a representative case where both BKbg and MKbg were present in the same patch of membrane. In contrast to the non-reversible inhibition of BKbg, it was evident that the activity of MKbg was persistent in the presence of ATP⁺S. The resistance of MKbg to MgATP was confirmed in eight patches (see also Fig. 6A). The concentration dependence of the inhibitory effects on BKbg was obtained from the decrease of the $P_o$ by various concentrations of MgATP, where the IC₅₀ was 0.18 mM (Fig. 5D).

Next, the inhibitory mechanism of MgATP was investigated. K-252a (400 nM), a nonspecific inhibitor of cAMP-dependent protein kinase, protein kinase C, protein kinase G, and calmodulin-dependent kinase at this concentration (23) did not block the effect of MgATP (Fig. 6A). Chelerythrin (5 μM), a protein kinase C inhibitor, also did not block the inhibition by MgATP (n/3, data not shown). The relatively high IC₅₀ value of ATP and the insensitivity to protein kinase inhibitors suggested that a lipid phosphorylation might mediate the inhibition of BKbg by MgATP. It was reported recently (24–26) that cytoplasmic application of ATP regulates various ion channels and transporters by generating PIP₂ in excised patch clamp conditions. Aluminum ion (Al³⁺) is known to form a highly stable complex with PIP₂ and blocks the PIP₂-dependent regulation ofchannels and transporters (25). In the i-o recording of BKbg, a pretreatment with Al³⁺ (50 μM) completely blocked the inhibitory effects of MgATP (n = 8, Fig. 6B). Also,
the pretreatment with wortmannin (50 μM), a PI-3 and PI-4 kinase inhibitor at this concentration, blocked the inhibitory effects of MgATP (n = 6, Fig. 6C). Finally, a direct application of PIP₂ (10 μM) to the cytoplasmic side (bath solution) suppressed the BKbg activity (n = 5, Fig. 6D). These results commonly suggest that the cytoplasmic ATP-dependent inhibition of BKbg is tightly related with PIP₂ in the membrane.

Effects of 2-APB on BKbg—The sensitivity of BKbg to MgATP strongly suggested that most of the whole-cell current, namely I_{Kbg}, was because of the activity of BKbg. Therefore, we tested whether 2-APB could also stimulate BKbg. In the c-a recordings of BKbg, a bath application of 2-APB (50 μM) induced a huge increase of P_o, which was reversed by washout of 2-APB (Fig. 7A). Similar responses to 2-APB were observed in more than 20 c-a recordings of BKbg.

In the i-o recordings without MgATP, the effect of 2-APB was hard to determine because the P_o of BKbg was already very high (n = 3, data not shown). Therefore, it was tested whether an application of 2-APB could overcome the inhibitory effects of MgATP on BKbg. In the presence of MgATP, the application of 2-APB (50 μM) to the cytoplasmic side induced a transient increase of P_o followed by a slight tonic increase (Fig. 7B, n = 5). Because the positive effect of 2-APB on BKbg was largely abolished, a cytosolic molecule mediating the effect of 2-APB might have been washed off in the i-o conditions. To test this hypothesis, we prepared a cytosolic fraction of WEHI-231 cells and applied it together with 2-APB. However, the co-application of cytosolic fraction (2.8 mg protein/ml) and 2-APB could not overcome the inhibitory effect of MgATP (Fig. 7C).
This study for the first time demonstrates background-type K⁺/H11001 channels (BK bg and MK bg) in lymphocytes. Besides their voltage-independent activity, BK bg displayed intriguing properties including the following: 1) conspicuous large unitary conductance (346 pS); 2) inhibition by cytoplasmic MgATP most likely mediated by phosphoinositide phosphorylation; and 3) facilitation by 2-APB. The responses of BKbg to MgATP and 2-APB strongly suggest that the whole-cell K⁺ current of WEHI-231, namely I K,bg, was largely due to the activity of BKbg. Because membrane hyperpolarization could provide a driving force for Ca²⁺, the activation of BKbg by 2-APB could explain our previous finding that 2-APB facilitates Ca²⁺ influx in WEHI-231 cells (16).

**Background-type K⁺ Channels in WEHI-231 Cells**—To our knowledge, the unitary conductance of the BKbg is larger than

---

**Fig. 5. Inhibition of BK bg activity by cytoplasmic application of MgATP.** The recordings were commonly obtained under the i-o patch clamp conditions at −60 mV with symmetrical KCl (145 mM) solutions. A, application of MgATP (1 mM) to the cytoplasmic side of membrane greatly reduced the activity of BK bg, which was completely reversed by the washout with control solution. Note the time break of 90 s. Histogram of \( P_o \) obtained from the experiment shown above (lower panel). The numbers in parentheses indicate the moment where displayed current traces were recorded, respectively. B, bath application of AMP-PNP (1 mM) had no effect on the activity of BK bg that was sensitively decreased by MgATP (1 mM). C, a representative case where BK bg and MK bg activities were concomitantly recorded (note two open states with different amplitudes). A bath application of ATPxS (1 mM) abolished the BK bg activity, whereas the MK bg was not affected. Note that the inhibition of BK bg by ATPxS is not reversed by washout. D, concentration-dependent effects of MgATP on BK bg activity. The \( P_o \) of BK bg measured at each concentration of MgATP was normalized to the control \( P_o \) (\( P_o,\text{con} \)), and the means ± S.E. were plotted, which was fitted by the function \( \frac{P_o}{P_o,\text{con}} \times 100 \text{ (%) } = 100(1 + \text{ (tested concentration/IC50)}) \).
any other classes of K⁺ channels including the large conductance Ca²⁺-activated K⁺ channels (maxi-K channels, 200–250 pS) (1). The involvement of maxi-K channels in the present study is highly unlikely because the cytoplasmic Ca²⁺ activity was clamped close to zero throughout the experiment, and iberiotoxin or TEA did not block the current. Moreover, the \( P_\infty \) of BKbg is voltage-independent, whereas the maxi-K channels are well known for their voltage-dependent increase of channel activity (1).

Leak or background-type K⁺ channels are defined by the lack of voltage dependence in channel activity. In this study, the \( P_\infty \) of BKbg is voltage-independent. Although the \( P_\infty \) of MKbg is about 2-fold higher at +60 mV than at −60 mV, such a difference is much weaker than classical voltage-gated K⁺

---

**FIG. 6. Mechanism of the inhibitory effects of MgATP on BKbg.** The recordings were commonly obtained under the i-o patch clamp conditions at −60 mV with symmetrical KCl (145 mM) solutions. 1 mM MgATP was applied. A, in the presence of K252a (400 nM), MgATP suppressed BKbg activity, whereas the MKbg was not affected. B and C, the application of \( \text{Al}^{3+} \) (50 μM) or wortmannin (50 μM) blocked the inhibitory effects of MgATP on BKbg. In some cases, \( \text{Al}^{3+} \) alone increased the activity of BKbg as demonstrated in B. D, the application of PIP₂ (10 μM) largely abolished the activity of BKbg.
channels or Ca\(^{2+}\) channels. The BK\(_{bg}\) and MK\(_{bg}\) in WEHI-231 cells commonly display weak inward rectification in terms of their unitary current to voltage relations. As a whole, such properties of single channel currents would be reflected as a linear or sublinear whole-cell current to voltage relation in symmetrical K\(^+/H\) gradient, as demonstrated in this study (Fig. 1E and Fig. 2).

The voltage-independent activity and resistance to TEA of IK\(_{bg}\) are similar to the traits of those K\(^+\) channels with two pore domains in tandem, called K2P channels (19, 20). However, the very large conductance of BK\(_{bg}\) and the inhibitory actions of intracellular MgATP are unprecedented in the K2P channels. Although IK\(_{bg}\) decreased moderately by acidification of extracellular fluid (pH 6.4), the contribution of TASK channels is unlikely considering a large difference in the single-channel conductance (19). The conductance of MgATP-insensitive MK\(_{bg}\) (112 pS) is quite comparable with that of TREK-2 channels (22). However, the inhibition of MK\(_{bg}\) by intracellular acidification and no response to membrane stretch are inconsistent with the properties of TREK-2 (22). These findings suggest that the background K\(^+\) channels in WEHI-231 cells, especially BK\(_{bg}\), represent novel classes of K\(^+\) channels, the molecular nature of which remains to be identified.

**Regulation of BK\(_{bg}\) Activity by Intracellular MgATP**

—Both whole-cell dialysis with MgATP-free solution and direct application of MgATP to i-o patches document the strong inhibitory

---

**Fig. 7. Effects of 2-APB on BK\(_{bg}\).** Representative cases of c-a recording (A) and i-o recordings (B and C) are shown. Holding voltage was −60 mV. A, bath application of 2-APB (50 μM, b and c) slowly increased the \(P_o\) of BK\(_{bg}\) which was reversed by washout of 2-APB. The lowermost panel shows a histogram of \(P_o\) plotted against the recording time. The lowercase letters indicate the moment where the above current traces were recorded. B, after confirming the inhibition of BK\(_{bg}\) activity by MgATP (1 mM, a), 2-APB (50 μM) was added to the cytoplasmic side, which induced a transient partial recovery of BK\(_{bg}\) activity (b). Lower panels show histograms of \(P_o\) obtained from the experiment shown above. Numbers in parentheses indicate the time where the above current traces were recorded. In the lowermost panel, a part of the above histogram was expanded to demonstrate the washout effect of 2-APB in the presence of MgATP (see also c of B). C, the addition of cytosolic fraction (2.8 mg of protein/ml) did not facilitate the effect of 2-APB (50 μM) on BK\(_{bg}\).
Regulation of Novel Background K⁺ Channels by MgATP

Regulation of Novel Background K⁺ Channels by MgATP—At a glance, the inhibition by intracellular ATP reminded us of the behavior of ATP-sensitive K⁺ channels (KATP) and Slick, a Na⁺-activated K⁺ channel inhibited by ATP (1, 21). However, glibenclamide (10 μM), a selective inhibitor of KATP, had no effect on IKbg (n = 3, data not shown). Also, neither the application of KATP openers (e.g. levromakalim, 5 μM) nor an increase in cytoplasmic Na⁺ (50 mM) affected BKbg activity (n = 4 and 5, respectively, data not shown). Because only hydrolyzable MgATP, and neither Na₂ATP nor AMP-PNP, is that 2-APB somehow decreases the level of cytoplasmic MgATP, and subsequently relieves the tonic inhibition of BKbg. Because 2-APB can block various ion channels permeable to divalent cations (17), an inhibition of Mg²⁺ influx by 2-APB might decrease the level of phosphorylatable ATP, namely MgATP in the cytosol. The exact target sites and action mechanism of 2-APB on BKbg needs further investigation.

Role of K⁺ Channels in the Signal Transduction of Immune Cells—Previous studies in T cells emphasized the role of voltage-gated K⁺ channel (Kv1.3) and the calcium-activated K⁺ channel (IKCa1, SK4) regulating the membrane potential and the Ca²⁺ signaling (2, 3, 8, 36). In T cells, the expression of IKCa1 is dramatically up-regulated by T cell receptor activation (3), explaining the stronger Ca²⁺ responses to secondary T cell receptor stimulation in activated T cells (37). In another study, however, repetitive stimulation of naive T cells induces terminally differentiated effector memory T cells with the characteristic expression pattern of high Kᵥ1.3 and low IKCa1 (38). Although studies on B cells are rare, intriguing similarities like the up-regulation of IKCa1 channels by BCR stimulation have been reported (7, 8). A recent study in DT-40 B cells has demonstrated that BCR cross-linking exerts inhibitory effects on the thapsigargin-induced SOCE, part of which is mediated by a membrane depolarization (39). In a similar context, the regulation of IKbg of WEHI-231 cells might play a role in the regulation of [Ca²⁺]i.

At the normal intracellular concentration of ATP in the intact B cells, it seems likely that the BKbg would be under the tonic inhibitory control. In fact, the Ps of BKbg under the c-a conditions was very low compared with their maximum capacity revealed after the excision of patch membrane (Fig. 3C). Considering their large unitary conductance, however, only a partial recruitment of BKbg might be sufficient to change the membrane voltage of B cells. As the BKbg activity was inhibited by PIP₂, a plausible mechanism of BKbg activation is a decrease of PIP₂. The BCR cross-linking and subsequent activation of phospholipase Cγ (9, 10, 14) would degrade PIP₂ to produce inositol 1,4,5-trisphosphate, which might facilitate BKbg activity in WEHI-231 cells. However, it is also documented that the net synthesis of PIP₂ is increased in B cells after BCR stimulation, which is signaled via Btk, a Tec family cytoplasmic tyrosine kinase (40). The physiological role and direction of BKbg regulation in the immature B cells will be an interesting theme to be pursued and should be interpreted depending upon the signaling pathways recruited.

In contrast to WEHI-231 cells, the IKbg was not found in mature B cells (Fig. 1F). Considering the apoptotic tendency of WEHI-231 cells, it would be an intriguing question whether an aberrant activation of IKbg might play a role in the cell death of immature B cells (9, 10). Persistent loss of potassium ions and the decrease of cell volume have been suggested as critical steps during cell death (1, 5, 6). In addition, roles of background-type K₂P channels have been suggested in the apoptosis of embryonic cells and cerebellar granule neuron (41, 42). Thus, it remains to be investigated whether the background K⁺ channels play a similar role in the apoptotic response of immature B cells to BCR stimulation.

In summary, we report a background-type K⁺ current (IKbg) and corresponding channels with unique properties (BKbg, MKbg) in WEHI-231, murine immature B lymphocytes. The activity of BKbg is sensitively regulated by cytosolic MgATP, which is most likely mediated by the level of PIP₂. Future investigations will be focused on the modulation of background K⁺ channels by immunological signals and their roles in the immature B cells.
REFERENCES

1. Ashcroft, F. M. (2000) *Ion Channels and Disease*, pp. 97–159, Academic Press, San Diego.

2. Verheugen, J. A., and Vijverberg, H. P. (1995) *Cell Calcium* 17, 287–300.

3. Ghanashy, S., Wulf, H., Miller, M. J., Rohm, H., Neben, A., Gutman, G. A., Cahalan, M. D., and Chandy, K. G. (2000) *J. Biol. Chem.* 275, 37137–37149.

4. Liu, Q.-H., Fleischmann, B. K., Hondowicz, B., Maier, C. C., Turka, L. A., Yui, K., Kolliker, M. I., Wells, A. D., and Freedman, B. D. (2002) *J. Exp. Med.* 196, 897–909.

5. Bortner, C. D., and Cidlowski, J. A. (2000) *Immunol. Rev.* 14, Marshall, A. J., Niiro, H., Yun, T. J., and Clark, E. A. (2000) *Immunology* 12, Tasker, L., and Marshall-Clarke, S. (2000) *Immunol. Rev.* 11, Igarashi, H., Kuwahara, K., Nomura, J., Matsuda, A., Kikuchi, K., Inui, S., and Sakaguchi, N. (1994) *J. Immunol.* 153, 2381–2393.

6. Yu, S. P., Canzoniero, L. M. T., and Choi, D. W. (2001) *Am. J. Physiol.* 28, 175, 3687–3691.

7. Partiseti, M., Choquet, D., Dis, A., and Korn, H. (1992) *J. Immunol.* 148, 3361–3368.

8. Lewis, R. S, and Cahalan, M. D. (1995) *Annu. Rev. Immunol.* 13, 623–653.

9. King, L. B., and Monroe, J. G. (2000) *Immunol. Rev.* 176, 86–104.

10. Kurokaki, T. (2002) *Curr. Opin. Immunol.* 14, 341–347.

11. Igashita, H., Kuwahara, K., Nomura, J., Matsuda, A., Kikuchi, K., Inui, S., and Sakaguchi, N. (1994) *J. Immunol.* 153, 2381–2393.

12. Tasker, L., and Marshall-Clarke, S. (2000) *Immunology* 99, 385–393.

13. Wu, Y., Pani, G., Siminosvitch, K. A., and Hozumi, N. (1995) *Eur. J. Immunol.* 25, 2279–2284.

14. Marshall, A. J., Nuro, H., Yun, T. J., and Clark, E. A. (2000) *Immunol. Rev.* 176, 30–46.

15. Parekh, A. B., and Penner, R. (1997) *Physiol. Rev.* 77, 901–930.

16. Nam, J. H., Yun, S. S., Kim, T. J., Uhm, D.-Y., and Kim, S. J. (2003) *FEBS Lett.* 535, 113–118.

17. Bootman, M. D., Collin, T. J., Mackenzie, L., Roderick, H. L., Berridge, M. J., and Poppiatt, C. M. (2003) *FASEB J.* 17, 1145–1150.

18. Braun, F.-J., Asir, O., and Putney, J. W., Jr. (2003) *Cell Calcium* 33, 1304–1311.

19. Lesage, F., and Lazdunski, M. (2000) *Am. J. Physiol.* 279, F9–F801.

20. Goldstein, S. A. N., Blochenerauer, D., O’Kely, J., and Zilberberg, M. (2001) *Nat. Rev. Neurosci.* 2, 175–184.

21. Bhattacharjee, A., Joiner, W. J., Wu, M., Yang, M., Sigworth, F. J., and Mok, C. (2003) *J. Neurosci.* 23, 11021–11031.

22. Bang, H., Kim, Y., and Kim, D. (2000) *J. Biol. Chem.* 275, 17412–17419.

23. Knose, B., and Hefi, F. (1992) *J. Neurochem.* 59, 897–1996

24. Hilgeman, D. W., Feng, S., and Nasuholgu, C. (2001) *Science’s STKE* http://www.stke.org/cgi/content/full/OC_stke/2001/11/RE11.

25. Hilgeman, D. W., and Ball, R. (1996) *Science* 273, 569–566.

26. Lee, M. Y., Bang, H. W., Lim, I. J., Uhm, D. Y., and Rhee, S. D. (1994) *Pfluegers Arch.* 429, 150–152.

27. Chemin, J., Girard, C., Duprat, F., Lesage, F., Romey, G., and Lazdunski, M. (2003) *EMBO J.* 22, 5403–5411.

28. Han, J., Kang, D., and Kim, D. (2003) *J. Physiol. (Lond.*) 150, 693–706.

29. Maryama, T., Kanaj, T., Nakade, S., Kanno, T., and Mikoshiba, K. (1997) *J. Biochem. (Tokyo)* 122, 498–505.

30. Bilmen, J. G., Wootton, L. L., Godfrey, R. E., Smart, O. S., and Michelangeli, F. (2002) *Eur. J. Biochem.* 269, 3678–3687.

31. Harks, E. G., Camina, J. P., Peters, P. H., Ypey, D. L., Scheene, W. J., van Zeele, E. J., and Thevenet, A. P. (2003) *FASEB J.* 17, 941–943.

32. Ma, H.-T., Venkatramakaram, K., Parry, J. B., and Grill, D. L. (2002) *J. Biol. Chem.* 277, 6015–6022.

33. Prakriya, M., and Lewis, R. S. (2001) *J. Physiol. (Lond.*) 536, 3–19.

34. Desai, R., Peretz, A., Idolson, H., Lazarovici, P., and Attali, B. (2000) *J. Biol. Chem.* 275, 29954–29963.

35. Verheugen, J. A., Le Deist, F., Devignot, V., and Korn, H. (1997) *Cell Calcium* 21, 1–17.

36. Wulf, H., Calabreshy, P. A., Allie, R., Yun, S., Pennington, M., Beetten, C., and Chandy, K. G. (2003) *J. Clin. Investig.* 111, 1703–1713.

37. Hashimoto, A., Hirose, K., Kurosaki, T., and Iino, M. (2001) *J. Immunol.* 166, 1003–1008.

38. Saito, K., Tolias, K. F., Saci, A., Koon, H. B., Humphries, L. A., Scharenberg, A., Rawlings, D. J., Kinet, J.-P., and Carpenter, C. L. (2003) *Immunity* 19, 669–678.

39. Trimarchi, J. R., Liu, L., Smith, P. J. S., and Keefe, D. L (2002) *Am. J. Physiol.* 282, C588–C594.

40. Lauritzen, I., Zanzouri, M., Honore, E., Duprat, F., Ehreng, M. U., Lazdunski, M., and Patel, A. J. (2003) *J. Biol. Chem.* 278, 32068–32076.
