The Effects of Shaker β-Subunits on the Human Lymphocyte K⁺ Channel Kv1.3*

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The activation of T-lymphocytes is dependent upon, and accompanied by, an increase in voltage-gated K⁺ conductance. Kv1.3, a Shaker family K⁺ channel protein, appears to play an essential role in the activation of peripheral human T cells. Although Kv1.3-mediated K⁺ currents increase markedly during the activation process in mice, and to a lesser degree in humans, Kv1.3 mRNA levels in these organisms do not, indicating post-transcriptional regulation. In other tissues Shaker K⁺ channel proteins physically associate with cytoplasmic β-subunits (Kvβ1–3). Recently it has been shown that Kvβ1 and Kvβ2 are expressed in mouse T cells and that they are up-regulated during mitogen-stimulated activation. In this study, we show that the human Kvβ subunits substantially increase K⁺ current amplitudes when coexpressed with their Kv1.3 counterpart, and that unlike in mouse, protein levels of human Kvβ2 remain constant upon activation. Differences in Kvβ2 expression between mice and humans may explain the differential K⁺ conductance increases which accompany T-cell proliferation in these organisms.

Voltage-gated K⁺ channels play an important role in the propagation of electrical signals in the nervous system of higher organisms (1). A large number of voltage-gated K⁺ channel proteins are expressed throughout the mammalian nervous system. These proteins are encoded by a large number of genes which fall into various families or subfamilies of homology. The mammalian Shaker family of K⁺ channels contains at least seven different genes, Kv1.1–Kv1.7 (2), which form functional homo- and heterotetrameric channel complexes (3, 4). Furthermore, it has been shown that in the mammalian nervous system the channel-forming Shaker proteins are usually complexed with cytoplasmic Kvβ subunits (Kvβ1–Kvβ3) (5).

Coexpression studies, utilizing mRNA injection and voltage-clamp analysis of Xenopus oocytes, have shown that the major brain Kvβ subunit (6), Kvβ2, and at least one splice form of Kvβ1 (Kvβ1a) are able to alter the inactivation properties of a number of neuronaly expressed Kv1 channel proteins (7, 8). In contrast, neither of these two Kvβ subunits significantly alters the inactivation properties of Kv1.3, a K⁺ channel that is sparsely expressed within the nervous system (9). Perhaps more importantly, Kvβ subunits are also able to increase K⁺ current amplitudes of neuronal Kv1 K⁺ channels in the oocyte plasma membrane (8). Furthermore, coexpression of several neuronal Kv1 channels with Kvβ2 in mammalian cell lines has shown that Kvβ2 increases the number of these Kv1 proteins reaching the membrane surface (10). Taken together, these studies indicate that the Kvβ subunits are likely to increase the surface expression of functional, neuronal K⁺ channels.

In addition to their role in the nervous system, K⁺ channel proteins are expressed in other cell types, where they may help determine membrane potential and maintain osmotic equilibrium. What specific physiological roles might these K⁺ channel proteins play outside the nervous system? They appear to play an essential role in the stimulation and maintenance of cellular proliferation of T cells (11), B cells (12), macrophages (13), and brown adipocytes (14). In T-lymphocytes, the role has been extensively investigated: mitogens cause an immediate shift in K⁺ conductance (15, 16); activated T cells show substantial greater K⁺ conductances than quiescent cells (11, 17); activation is attenuated by membrane depolarization (18); and pharmacological agents that inhibit K⁺ channel conductances block T-cell proliferation (19). In human peripheral T-lymphocytes, the Kv1.3 K⁺ channel plays a critical role in mediating the K⁺ current increase, which accompanies proliferation (20), and is also likely to be the site of blockade, which inhibits this event (21). Interestingly, Kv1.3 mRNA does not appear to be up-regulated during proliferation, indicating that the Kv1.3 gene product is post-transcriptionally regulated (22, 23).

Recent studies have shown that Kvβ2, and Kvβ1 to a lesser extent, are both expressed in mouse T-lymphocytes (24). Moreover, the murine Kvβ mRNA and protein levels are markedly increased upon interleukin-2 stimulation. If the Kvβ subunits were able to increase the surface expression of Kv1.3 channels, then up-regulation of Kvβ subunits would result in greater K⁺ channel surface expression and therefore greater K⁺ conductance during the proliferation of T-lymphocytes and perhaps other cell types (11–14). The extent of Kvβ subunit up-regulation in response to T-cell activation could therefore account for the extent to which Kv1.3-mediated K⁺ current is elevated in different organisms. Utilizing the Xenopus oocyte expression system, we report the effects of coexpression of the human Kvβ1a and Kvβ2 subunits on the expressed current levels of Kv1.3 channels.

**EXPERIMENTAL PROCEDURES**

In Vitro Transcription and Oocyte Injection—The Kvβ1 and Kvβ2 cDNAs were subcloned into a vector containing 40 adenosine residues downstream of the 3′ cloning site and linearized with NotI. The β1 cDNA had 10 nucleotides 3′ of the stop codon. The human Kv1.3 gene was transcribed from linearized template. For more efficient expression in oocytes (8), the 5′ untranscribed sequences immediately upstream of the ATG start codon of Kvβ2 and Kv1.3 were changed to GCCGCCCAAG. Oocytes were injected with 50 nl of cRNA at varying dilutions. All electrophysiology experiments were carried out at approximately 20 °C.
Microinjection and Electrophysiology—Ovaries of specimens of *Xenopus laevis* were surgically removed, and individual oocytes were dissec
ted away in a saline solution (ND96) containing 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 2 mM MgCl₂, and 5 mM HEPES at pH 7.4 with NaOH. Stage V and VI oocytes were treated for 2 h with collagenase (1 mg/mL) at 37 °C to discard follicle cells. cDNA solutions of 50 nl were injected into oocytes using the Nanoject injector apparatus (Drummond Scientific, Broomall, PA). Measurement of ionic currents in *Xenopus* oocytes was performed using standard two-electrode voltage clamp techniques. Recordings were obtained with the GeneClamp500 voltage clamp and the PCLAMP software package (Axon Instruments, Foster City, CA). Electrodes of 0.4–1.0 megohms were filled with 1 M KCl. Recordings were conducted in ND96 solution. Data were leak subtracted using hyperpolarizing F/4 subtraction pulses from a holding potential of −80 mV. The interpulse interval was 25 s. K⁺ conductances were calculated using a reversal potential of −80 mV.

Cell Culture—Peripheral blood mononuclear cells were isolated by Ficoll Gradient and then added to media containing 10% fetal calf serum, 100 units/ml penicillin, 100 mg/ml streptomycin, and 100 units/ml interleukin-2. One-half of the cells were frozen at −80 °C immediately after separation and thawed into media. The other cells were activated with phytohemagglutinin at 5 μg/ml at 37 °C. Cells were activated for 72 h.

Western Blot Analysis—Human T-lymphocytes were treated with or without phytohemagglutinin as described above. These cells were then homogenized in RIPA buffer (25 mM Tris, pH 7.4, 150 mM KCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS). Proteins were size-fractionated on a 9% SDS-polyacrylamide gel and then transferred to Nitrocellulose membrane. The membranes were blocked in 100 mM phosphate-buffered saline containing 10% nonfat dried milk and 0.05% Tween-20. The anti-Kvβ2 polyclonal antibody (Quality Controlled Biochemicals, Inc., Hopkinton, MA) raised against the C-terminal 18 amino acids of the Kvβ2 protein was recognized by an anti-rabbit IgG secondary antibody. Membranes were washed in 100 mM phosphate-buffered saline containing 3% dried milk and 0.05% Tween-20. Immunoreactivity was visualized by a horseradish peroxidase-catalyzed color reaction (Pierce, Rockford, IL).

RESULTS

*Kv1.3 Expression*—Representative current traces of uninjected oocytes (Fig. 1A) and oocytes injected with 0.2 ng of Kv1.3 cRNA (Fig. 1B) are shown. The time course of Kv1.3 expression in the absence of β-subunits was observed. Recordings at +60 mV were taken at 18, 24, 36, 48, and 72 h after injection. Kv1.3 channels expressed a slowly inactivating current (τ = 548 ms at +60 mV ± 38 ms, n = 8). The current amplitude of Kv1.3 continued to increase with increasing time, with levels still increasing up to 72 h after injection (Fig. 1C).

For all of the experiments reported below, Kv1.3 RNA was injected into *Xenopus* oocytes at concentrations, approximately 0.2 ng/oocyte, which produced relatively small currents. At concentrations as much as 10-fold higher, the relationship between the amount of RNA injected and current amplitudes is linear, suggesting that cellular translation and transport functions are not saturated in this concentration range (Fig. 1D).

**Functional Expression of α1.3β2K⁺ Channel Complexes**—To investigate the potential effects of the β-subunits on Kv1.3 current, we first co-injected Kv1.3 with varying amounts of Kvβ2 cRNA. We observed that the enhancement of Kv1.3 expression increased markedly until the β2 cRNA level approached 10-fold that of the Kv1.3 cRNA (Fig. 2A). Beyond that ratio, there was little increase in the enhancement of current, suggesting that saturating amounts of the Kvβ2 subunit had been reached.

*Xenopus* oocytes were injected with Kv1.3 cRNA and saturating amounts of Kvβ2 cRNA. Recordings were taken at 18, 24, 48, and 72 h after injection. At all time points investigated, there was a striking increase in the current amplitude of Kv1.3 when co-expressed with Kvβ2 (Fig. 2B). The increase in current of Kv1.3 in the presence of saturating amounts of Kvβ2 was 4-fold (±0.38, n > 10) at 18 h, 4.2-fold (±0.45, n > 10) at 24 h, 3.2-fold (±0.38, n > 10) at 48 h, and 2.6-fold (±0.44, n > 10) at 72 h (Fig. 2B). Marked increases in current were obtained when at least equal amounts of Kv1.3 and Kvβ2 cRNA were used. Unlike Kv1.4, the inactivation of Kv1.3 was not accelerated when coexpressed with Kvβ2 (Fig. 3, A and C). Scaled currents of Kv1.3 alone (Fig. 4A) and Kv1.3 with Kvβ2 (Fig. 4B) show that kinetics of Kv1.3 are not significantly altered by the presence of Kvβ2. Plots of the normalized voltage-conductance relationships (Fig. 5) rule out the possibility that the Kvβ-mediated increases in current amplitude are because of alterations...
in the voltage-gating properties of Kv1.3 channels; the probability of opening is saturated at approximately +20 mV both in the absence and presence of the Kvβ subunits.

**Functional Expression of α1.3β1,2 Channel Complexes**—We co-injected *Xenopus* oocytes with Kv1.3 cRNA and saturating amounts of Kvβ1 cRNA. Unlike most other Kv1 channels, Kv1.3 did not exhibit rapid inactivation when co-expressed with Kvβ1 (Fig. 3B), though controls performed with Kv1.4 and Kvβ1 confirmed that the β1 cRNA was being translated properly (data not shown). We observed an increase in current amplitude when Kv1.3 was co-expressed with Kvβ1, peaking at slightly more than 2-fold at 24 h, but by 72 h after injection the Kv1.3 current amplitude in the presence of Kvβ1 decreased to 67% of the current of Kv1.3 injected alone (Fig. 2B). The voltage-dependence of Kv1.3 was not affected by the presence of the Kvβ1 subunit (Fig. 5). Taken together, the data indicate that although the Kvβ subunits do not alter gating or inactivation, they strongly regulate the expression of functional channels at the cell surface membrane.

**Kvβ2 Subunit Protein Expression**—A polyclonal antibody against Kvβ2 was used to detect the expression of β-subunits in T-lymphocytes. In both quiescent and activated T-lymphocytes, a 39-kDa protein is detected at levels which remain relatively constant (Fig. 6). This 39-kDa protein is identical in size to the Kvβ2 channel subunit purified from bovine brain (6) and the Kvβ2 subunit detected in activated murine T-lymphocytes (24). A faint band just below the 39-kDa band in Fig. 6 is likely to correspond to a previously reported splice variant of Kvβ2 (GenBank accession number 2827466), in which 14 amino acids near the N terminus of the protein are absent. In contrast to murine T-lymphocytes, the expression levels of Kvβ2 do not change in activated human T-cells.

**DISCUSSION**

T-lymphocytes are critical for eliciting cellular immune responses. It is well established that K⁺ channels play important roles in the activation of T-lymphocytes: important physiologic changes which T-lymphocytes undergo as a result of the activation process are inhibited by blockers of K⁺ channels, including protein synthesis, cell volume increase, and cell-cycle progression (17, 19). It has been demonstrated that channels containing the Kv1.3 subunit are the major K⁺ channel (type n channel) in T-lymphocytes (25). The K⁺ conductance increases 20-fold in murine T-cells treated with mitogen, whereas in human T-cells, the K⁺ conductance increases roughly 2-fold (11, 17). However, treatment of these cells by a mitogen results in constant or decreased, rather than increased, Kv1.3 mRNA levels in mice and humans, respectively (22, 23). On the other hand, it has been shown that the expression of the murine Kvβ2 subunit increases more than 2-fold in response to stimulation by interleukin-2 (24). Our results show that Kv1.3 can form functional channels alone but that the presence of Kvβ subunits, primarily Kvβ2, the more abundant β-subunit in human T-lymphocytes (24), accelerates the functional assembly of Kv1.3 channels. The ability of the Kvβ2 subunit to enhance current levels of Kv1.3 may help to explain how the increase in Kvβ2 subunit expres-
FIG. 4. Kvβ2 does not affect Kv1.3 current kinetics. Shown is the family of K⁺ currents elicited by 2-s pulses from −60 to +60 mV in 10-mV increments from a holding potential of −80 mV, 48 h after injection. Currents elicited by Kv1.3 alone (A) and Kv1.3 with saturating amounts of Kvβ2 cRNA (B). Current amplitudes are scaled to equal heights. The mean conductance values were normalized to the maximum conductance, and the mean ± S.E. were plotted against the depolarization potential. Kv1.3 (circles), Kv1.3 with Kvβ1 (triangles), and Kv1.3 with Kvβ2 (squares).

FIG. 5. Voltage-dependence of Kv1.3 with and without β-subunits. Conduction voltage relationships were determined by depolarizing from a holding potential of −80 to pulses ranging from −60 to +60 mV in 10-mV increments from Xenopus oocytes 24 h after injection. Conductance values were normalized to the maximum conductance, and the mean values ± S.E. were plotted against the depolarizing potential. Kv1.3 (circles), Kv1.3 with Kvβ1 (triangles), and Kv1.3 with Kvβ2 (squares).

FIG. 6. Western blot analysis of Kvβ2 protein expression in quiescent and activated human T-lymphocytes. Cell extracts were analyzed for Kvβ2 protein levels by Western blot analysis. Lane 1, unstimulated human T-cells; lane 2, T-cells stimulated for 72 h with phytohemagglutinin (5 µg/ml). 100 µg of protein were added to both lanes.

...regulation in mice and humans may account for the vast difference in the extent to which Kv1.3 K⁺ conductance is up-regulated in activated T-cells in these two organisms.

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REFERENCES
1. Rudy, B. (1988) Neuroscience 25, 729–749
2. Coetere, W., Amarillo, Y., Chiu, J., Chow, A., Lau, D., McCormack, T., Moreno, H., Nadal, M. S., Ozaita, A., Pountney, D., Saghani, M., Vega-Saenz de Miera, E., and Rudy, B. (1999) Ann. N. Y. Acad. Sci. 868, 233–285
3. McCormack, K., Lin, J. W., Iverson, L. E., and Rudy, B. (1990) Biochem. Biophys. Res. Commun. 171, 1361–1371
4. Ruppersberg, J. P., Schroter, K. H., Sakmann, B., Stocker, M., Sewing, S., and Pungs, O. (1990) Nature 345, 535–537
5. Shamotoieno, O. G., Parej, D. N., and Dolly, J. O. (1997) Biochemistry 36, 8195–8201
6. Scott, V. E. S., Rettig, J., Parej, D. N., Keen, J. N., Findlay, J. B. C., Pungs, O., and Dolly, J. O. (1994) Proc. Natl. Acad. Sci. 91, 1637–1641
7. Rettig, J., Heinemann, S. H., Wunder, F., Lorra, C., Parej, D. N., Dolly, J. O., and Pungs, O. (1994) Nature 369, 289–294
8. McCormack, K., McCormack, T., Tanouye, M., Rudy, B., and Stuhmer, W. (1995) FEBS Lett. 370, 32–36
9. Heinemann, S., Rettig, J., Scott, V., Parej, D. N., Lorra, C., Dolly, J., and Pungs, O. (1994) J. Physiol. (Parat.) 88, 173–180
10. Shi, G., Nakahira, K., Hammond, S., Rhodes, K. J., Schechter, L. E., and Trimmer, J. S. (1996) Neuron 16, 843–852
11. Decoursey, T. E., Chandy, K. G., Gupta, S., and Cahalan, M. D. (1987) J. Gen. Physiol. 89, 379–404
12. Sutro, J. B., Yavuvalara, B. S., Gupta, S., and Cahalan, M. D. (1989) Adv. Exp. Med. Biol. 254, 113–122
13. Kitagawa, S., and Johnston, R. B., Jr. (1985) J. Immunol. 138, 327–331
14. McKinnon, D., and Ceredig, R. (1986) J. Physiol. (Lond.) 199, 257–275
15. Lee, S. C., Sahab, D. E., Deutsch, C., and Prystowsky, M. (1986) J. Cell Biol. 102, 1202–1208
16. Gelfand, E. W., Cheung, R. K., Mills, G. B., and Grinstein, S. (1987) J. Immunol. 138, 527–531
17. DeCoursey, T. E., Chandy, K. G., Gupta, S., and Cahalan, M. D. (1984) Nature 310, 455–460
18. Decoursey, T. E., Chandy, K. G., Gupta, S., and Cahalan, M. D. (1987) J. Gen. Physiol. 89, 455–460
19. Koo, G. C., Blake, J. T., Talento, A., Nguyen, M., Lin, S., Sirotina, A., Shah, K., Mulvaney, K., Hara, D., Jr., Cunningham, P., Wunderler, D. L., McManus, O. B., Slaughter, R., Bugiani, R., Felix, J., Garcia, M., Williamson, J., Kazcorowiski, G., Sigal, N. H., Springer, M. S., and Feeney, W. (1997) J. Immunol. 158, 5120–5128
20. Cai, Y. C., Osborne, P. B., North, K., Wunderler, D. L., McManus, O. B., Slaughter, R., Bugiani, R., Felix, J., Garcia, M., Williamson, J., Kazcorowiski, G., Sigal, N. H., Springer, M. S., and Feeney, W. (1997) J. Immunol. 158, 5120–5128
21. Cai, Y. C., Osborne, P. B., North, R. A., Dooley, D. C., and Douglass, J. (1992) DNA Cell Biol. 11, 163–172
22. Attali, B., Romey, G., Honore, E., Schmid-Aliana, A., Mattei, M. G., Lesage, F., Ricard, P., Barhanin, J., and Lazdunski, M. (1992) J. Biol. Chem. 267, 8650–8657
23. Attali, B., Romey, G., Honore, E., Schmid-Aliana, A., Mattei, M. G., Lesage, F., Ricard, P., Barhanin, J., and Lazdunski, M. (1992) J. Biol. Chem. 267, 8650–8657
24. Atulier, M. V., Belkowski, S. M., Constantinescu, C. S., Cohen, J. A., and Prystowsky, M. B. (1997) J. Neuroimmunol. 8, 111
25. Lewis, R. S., and Cahalan, M. D. (1995) Annu. Rev. Immunol. 13, 623–654