The Involvment of Ion Channels in Cell Proliferation

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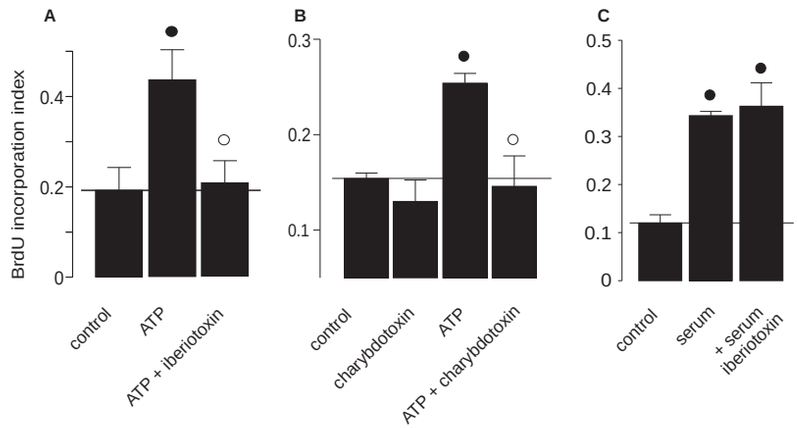
The control of cell proliferation involves diverse signaling pathways, growth factors, and receptors. The involvement of ion channels in this process is supported by a wealth of experimental evidence with mechanisms not always well understood. However, the emerging roles ion channels play in such cellular processes are related to changes in Ca^{2+} signaling, stressing its importance in proliferation initiation and maintenance. The use of specific ion channel blockers and antibodies enables researchers to unravel the complexities of this issue. Below we highlight the use of Alomone Labs' ion channel antibodies and modulators in some aspects related to this research field.

The control of cell proliferation involves diverse signaling pathways, growth factors, and receptors. The involvement of ion channels in this process is supported by a wealth of experimental evidence. Growth factors increase expression of K^+ channels.[1] Primary culture of bone marrow derived marcrophages is a unique non transformed model in which proliferation and activation can be studied separately.[2] In this system, the presence of $K_v1.3$ and $K_{ir}2.1$ was shown using immunocytochemical electron microscopic detection using **Anti-K_v1.3** antibody (#APC-002) and **Anti-K_{ir}2.1** antibody (#APC-026). The number and density of these channels was upregulated by treatment with M-CSF (the specific growth factor for this cell type) as shown in immunoblots using the above antibodies. This proliferation was inhibited by the $K_v1.3$ specific blocker, **rMargatoxin** (#RTM-325). A wide variety of mitogenic factors activate the Na/H exchanger, and many factors stimulate Na-K-2Cl cotransport.[3] One expected consequence of the activation of these transport systems is an increase of cell volume. Expression and single channel activity of some ion channels has been shown to vary during the cell cycle,[4] most notably during the transition from G0 or G1 to S phase. Czarnecki et al.[5] have shown that the peak density of the transient outward K^+ current (I_{to}) expression in the GH3 pituary cell is upregulated in quiescent cells compared with proliferating cells. Increase in I_{to} expresssion is accompanied by an increase in $K_v1.4$ alpha subunit protein levels, a decrease in $K_v1.5$ protein levels and no change in $K_v4.3$ protein levels. This was shown by Western blot using antibodies specific for each subunit: **Anti-K_v1.4** antibody (#APC-007), **Anti-K_v1.5** antibody (#APC-004) and **Anti- K_v4.3** antibody (#APC-017).

The functional contribution of $K_v4.3$ to the I_{to} current was confirmed using **Phrixotoxin** (#P-700), a specific blocker of $K_v4.3$ channels. As chloride channels are criticial to the cell cycle in vascular smooth muscle cells, the identification of the specific channel involved is of interest. Wang et al.[6] used **Anti-ClC3** antibody (#ACL-001) to show expression of ClC3 in VSMCs and functional enhancement by endothelin-1.

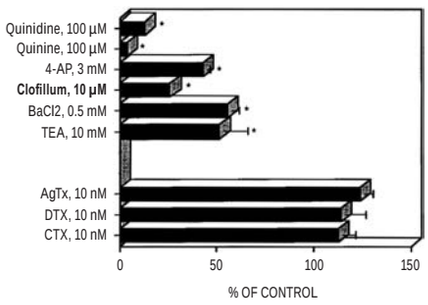
Cell proliferation must, at some point, increase cell volume since all cell components must be duplicated prior to cell division. Changes in cell volume require participation of ion transport[1] and rearrangements of the cytoskelton.[7] Cell proliferation has been shown to correlate with increases in cell volume in fibroblasts,[8] mesangial cells,[9] lymphocytes,[10] HL-60 cells,[11] GAP A3 hybridoma cells,[12] smooth muscle cells,[13] and HeLa cells.[14] In human T-lymphocytes $K_v1.3$ is involved in volume regulation since blocking the channel with **rAgitoxin-2** (#RTA-420) increased the maximal swelling of the cells.[15] Depolymerization of actin filaments leads to disinhibition of the

Inhibition of ATP-Induced DNA Synthesis by Blockers of BK Channels



ATP (500 µM) induced DNA synthesis was inhibited by blockers of BK channels. (A) **Iberiotoxin** (#RTI-400) (70 nM) reversed the mitogenic effect of ATP. (B) **Charybdotoxin** (#RTC-325) (100 nM) inhibited the ATP effect. (C) Iberiotoxin (100 nM) did not prevent the increase of the DNA synthesis evoked by fetal calf serum (5%). Data are the mean of 3 to 11 independent experiments. Adapted from Reference #22, with kind permission of Dr. Bringmann of the Paul Flechsig Institute of Brain Research, Department of Neurophysiology, University of Leipzig, and the *Invest. Ophthalmol. Vis. Sci.*.

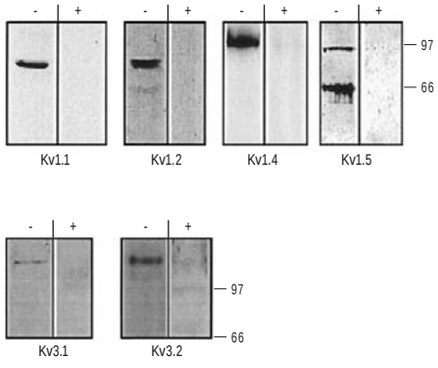
Effect of K_V Channel Blockers on Schwann Cell (SC) Proliferation



Proliferation was assayed in cultured SCs exposed for 24 hr to various channel blockers: **Charybdotoxin** (#RTC-325), **Dendrotoxin I** (#D-390), **Agitoxin-2** (#RTA-420) at the indicated concentrations in the presence of 10% FCS. The results were expressed as percentage of maximal serum-stimulated proliferation. Data points represent the mean 6 SEM of four independent experiments, each performed in triplicate.

Adapted from Reference #26 with the kind permission of Dr. Attali of the Department of Neurobiology, The Weizmann Institute of Science and the *J. Neurosci.*

Western Blot Analysis of K_V Channel Subunits in Cultured Schwann Cells (SC)



Membrane fractions of mouse primary cultured SCs were subjected to immunoblot analysis with subunit-specific antibodies: **Anti- K_V1.1** (#APC-009), **Anti-K_V1.2** (#APC-010), **Anti-K_V1.4** (#APC-007), **Anti-K_V1.5** (#APC-004), **Anti-K_V3.1b** (#APC-014), **Anti-K_V3.2** (#APC-011) α-subunits and antibody to K_V2.1. To check the specificity of antibody labeling toward SC membrane extracts, each antiserum was preincubated for 1 hr at room temperature in the presence (+) or absence (-) of its respective antigen. HRP-conjugated secondary antibodies and ECL were used for detection.

Adapted from Reference #26 with the kind permission of Dr. Attali of the Department of Neurobiology, The Weizmann Institute of Science and the *J. Neurosci.*

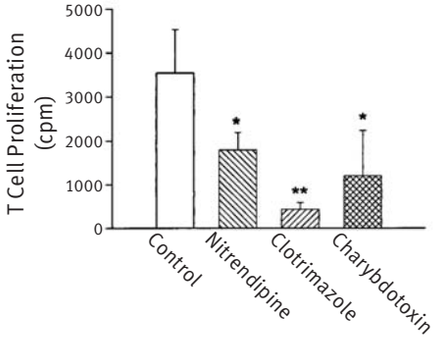
Na/H exchanger and/or Na-K-2Cl transporter, resulting in cell swelling.[16] Notably, increased channel activity is found in cancer cells.[17] Pillozzi et al.[18] used **Anti-hK$_V$11.1 (HERG)** antibody (#APC-062) to detect K_V11.1 (HERG) channels in the leukemic cell line FLG-29.1 and in primary leukemic cells. They further showed that a specific K_V11.1 inhibitor caused a strong inhibition of colony formation when the cells were seeded in semisolid medium. The presence of K_V11.1 protein was also shown in four hematopoetic cell lines, CEM, K562, U937 and HL-60 by Smith et al.,[19] using Anti-hK$_V$11.1 (HERG) antibody. They also showed that by blocking the K_V11.1 current with **E-4031** (#E-500) significantly decreased the number of cells.

The precise mechanisms by which ion channels regulate proliferation are not understood. Two major hypotheses attempt to explain this mechanism: regulation of membrane potential and regulation of cell volume. Phosphorylation/dephosphorylation has also been implicated.[20] K^+ channels maintain the membrane potential at negative values, providing a large driving force for calcium entry into cells. The elevated $[Ca^{2+}]_i$ (the Ca^{2+}concentration generated by calcium influx) is required for the cell's ability to proliferate.[21] In Muller cells, the activity of calcium dependent postassium channels (BK) was connected to the mitogenic action of ATP. Blocking these channels using **rCharybdotoxin** (#RTC-325) and **rIberiotoxin** (#RTI-400) fully inhibited the effect of ATP on DNA synethesis.[22] Membrane potential also regulates transport of (sodium-coupled) nutrients, which also affects the cell's ability to proliferate. According to the volume hypothesis,[23] K^+ channel activity controls the ion influx-efflux ratio and cell volume. If secondary volume-regulating mechanisms are not activated, K^+ channel blockade induces cell swelling. In turn, cell swelling dilutes the intracellular concentration of a solute controlling the expression or activity of genes or enzymes involved in DNA synthesis. As a corollary of this hypothesis, any change in cell volume should be associated with a change in cell proliferation. Whether the proliferative effect is a consequence of a change in ion channel number or a change in open probability, must be considered as well, although most studies have not explored this point.[24] In addition, the differences between excitable and non-excitable cells must be examined; the control of proliferation in cells that can produce an action potential may not be the same as in other cells. The control of proliferation in neoplastic cells may be governed by ion channels not expressed in their normal counterparts.

It should be noted that although both membrane potential and cell volume are regulated by ion channels, their relationship need not be linear; cell swelling can be induced by agents that do not affect membrane potential.[25] In addition, cell proliferation and changes in membrane potential/cell volume occur on different time scales – days vs. hours or shorter, respectively.

The effect of specific channel blockers on cell proliferation is cell type-specific. Charybdotoxin neither modified membrane current at a holding potential of –70 mV, nor affected cell volume, so it is not surprising that it did not block proliferation of C6 glioma cells.[23] However, in T-cells, rCharybdotoxin fully inhibited IK current[24] and blocked cell proliferation by 67%. In porcine granulosa cells, in contrast, inhibition of K_V1.3 with rCharybdotoxin and rMargatoxin caused an increase in cell proliferation when the cells were concomitantly treated with FSH (follicle stimulating hormone).[25] In these cells, the depolarization associated with rCharybdotoxin and rMargatoxin inhibition of K_V1.3 *enhances* calcium influx (in most cells, blockade of K^+ channels decreases calcium influx), thus increasing cell proliferation. This seems to be dependent upon the type of Ca^{2+}-conducting channels available in a particular cell, whether they are voltage-dependent or constitutive. Scwann cells have been shown to contain a variety of K^+ channels by the use of specific antibodies: **Anti-K_V1.1** antibody (#APC-009), **Anti-K_V1.2** antibody (#APC-010), Anti-Kv1.4 antibody, Anti-Kv1.5 antibody , **Anti-K_V3.1** antibody (#APC-014) and **Anti-K_V3.2** antibody (#APC-011). The expression of these channels was shown to be developmentally regulated. The specific blockers rCharybdotoxin, rAgitoxin-2 and **Dendrotoxin-I** (#D-390) did not affect cell proliferation, whereas non-specific blockers such as TEA, barium and clofilium did.[26]

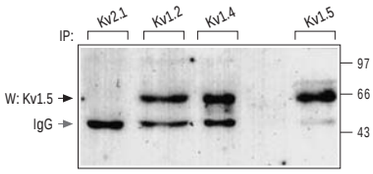
Effect of Nitrendipine, Clotrimazole, and Charybdotoxin on T Cell Proliferation



Cells were incubated for 5 days in culture medium with *Candida albicans* and the IK channel blockers (10 mM nitrendipine, 100 nM **rCharybdotoxin** (#RTC-325), or 10 mM clotrimazole), which were added 30 min before the addition of Candida albicans. [^{3}H]Thymidine (1 mCi) incorporation was then measured in triplicate wells. The bars represents 9 independent experiments 6 SE for control, and 3 independent experiments in the presence of Nitrendipine, Clotrimazole, and Charybdotoxin, respectively.

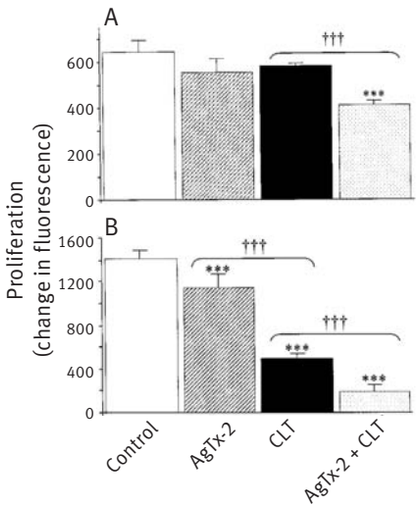
Adapted from Ref. # 24, with kind permission of Dr. Jensen, NeuroSearch A/S and the *Proc. Natl. Acad. Sci. USA.*

Co-Immunoprecipitation of K_v1.5 with Other Shaker-Like Subunits in Cultured Schwann Cells (SC)



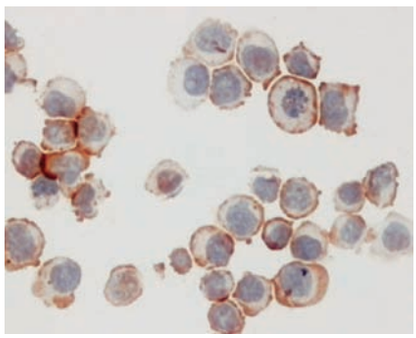
The heteromultimeric association of K_v1.2, K_v1.4, and K_v1.5 α subunits in cultured Schwann cells. Coimmunoprecipitation of K_v1.5 with other *Shaker*-like subunits. SC membranal fractions were subjected to immunoprecipitation with anti-K_v2.1, **Anti-K_v1.2** (#APC-010), **Anti-K_v1.4** (#APC-007), or **Anti-K_v1.5** (#APC-004) antibodies, and blots were probed with anti-K_v1.5 antibodies. Nonspecific bands corresponding to immunoglobulin heavy chain (*IgG*) are also observed. Adapted from Reference #26 with the kind permission of Dr. Attali of the Department of Neurobiology, The Weizmann Institute of Science and the *J. Neurosci.*

K+ Channel Blockers Inhibit Proliferation of Naive T Cells and Previously Activated T Lymphoblasts



Comparison of the ability of K+ channel blockers to inhibit proliferation of naive T cells (A) and previously activated T lymphoblasts (B) The term, naive, is used to indicate cells that were stimulated from an initial resting state. Each well of a 96-well plate was seeded with 2x10^4 resting cells or lymphoblasts and incubated with or without channel blockers for 10 min (5 nM **rAgitoxin-2** (#RTA-420), AgTx-2; 250 nM Clotrimazole, *CLT*). Then PHA-P (7 mg/ml) was added to initiate or restimulate proliferation. After 72 h, the CyQUANT assay was used to measure a change in fluorescence that is proportional to the change in cell number. Data are expressed as mean S.D. of four independent experiments (four replicates each). A Bonferroni multiple comparison test was used to assess each combination of treatments. Values that differ significantly from controls are indicated (***, p , 0.001), as are significant differences between drug treatments (†††, p , 0.001). Adapted from Ref. #15, with the kind permission of Dr. L.C. Schlichter of the Toronto Western Hospital and the *J. Biol. Chem.*

HERG Protein Detection in Leukemic Cell Line FLG 29.1



Immunocytochemical staining of FLG 29.1 leukemic cells with **Anti-hK$_v$11.1(HERG)** antibody (#APC-062) directed against the C-terminus of HERG. Adapted from Ref. #18 with the kind permission of Dr. A Arcangeli, Department of Experimental Pathology and Oncology, University of Firenze.

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Anti-CLC-2	ACL-002
Anti-CLC-3	ACL-001
Anti-CLC-5	ACL-003
Anti-CLC-K	ACL-004
Antibodies to Voltage Activated K+ Channels	
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Anti-K_v1.2	APC-010
Anti-K_v1.3	APC-002
Anti-K_v1.3 (extracellular)	APC-101
Anti-K_v1.3 (extracellular)-FITC	APC-101-F
Anti-K_v1.4	APC-007
Anti-K_v1.5	APC-004
Anti-K_v1.6	APC-003
Anti-K_v1.7	APC-063
Anti-K_v2.1	APC-012
Anti-K_v2.2	APC-120
Anti-K_v3.1b	APC-014
Anti-K_v3.2	APC-011
Anti-K_v3.3	APC-102
Anti-K_v3.4	APC-019
Anti-K_v4.1	APC-119
Anti-K_v4.2	APC-023
Anti-K_v4.3	APC-017
Anti-K_v7.1 (KCNQ1)	APC-022
Anti-K_v7.2 (KCNQ2)	APC-050
Anti-K_v7.3 (KCNQ3)	APC-051
Anti-K_v10.1 (EAG-1)	APC-104
Anti-KV10.2 (EAG-2)	APC-053
Anti-K_v11.1 (erg1)	APC-016
Anti-hK$_v$11.1 (HERG)	APC-062
Anti-K_v11.1 (HERG) (extracellular)	APC-109
Anti-K_v11.1 (HERG) (extracellular) FITC	APC-109-F
Anti-K_v11.2 (erg2)	APC-114
Anti-K_v11.3 (erg3)	APC-112
Anti-K_v12.1 (Elk1)	APC-113
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Anti-K_{ir}2.1	APC-026
Anti-K_{ir}2.2	APC-042
Anti-K_{ir}2.3	APC-032
Anti-K_{ir}3.1	APC-005
Anti-K_{ir}3.2	APC-006
Anti-K_{ir}3.3	APC-038
Anti-K_{ir}3.4	APC-027
Anti-K_{ir}4.1	APC-035
Anti-K_{ir}4.2	APC-058
Anti-K_{ir}6.1	APC-105
Anti-K_{ir}6.2	APC-020
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rAgitoxin-3	RTA-390
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BDS-I	B-400
BDS-II	B-450
rBeKm-1	RTB-470
rCharybdotoxin	RTC-325
α-Dendrotoxin	D-350
β-Dendrotoxin	D-360
γ-Dendrotoxin	D-370
δ-Dendrotoxin	D-380
Dendrotoxin-I	D-390
Dendrotoxin-K	D-400
E-4031	E-500
rErgtoxin-1	RTE-450
rHeteropodatoxin-2	RTH-340
rHongotoxin-1	RTH-400
rIberiotoxin	RTI-400
rKaliotoxin-1	RTK-370
rLq2	RTL-550
MCD-Peptide	M-250
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rMaurotoxin	RTM-340
rNoxiustoxin	RTN-340
rOsK-1	RTO-150
Paxilline	P-450
Penitrem A	P-650
Phrixotoxin-2	P-700
Stromatoxin-1 (rScTx-1)	RTS-350
rScyllatoxin	RTS-370
rSlotoxin	RTS-410
Stichodactyla Toxin (ShK)	S-400
rTamapin	RTT-400
rTertiapin	RTT-250
rTertiapin-Q	RTT-170
rTityustoxin Kα	RTT-360