Ion Channels in Cancer

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Introduction

Ion channels have long been known to be involved in the regulation of a variety of biological functions ranging from the control of cell excitability to the regulation of cell volume and proliferation. Because of the ubiquitous presence of ion channels in virtually all cells and their critical involvement in diverse biological functions, it came as no surprise when several human and animal diseases were attributed to defects in ion channel function. Indeed, the term channelopathies was coined to describe the ever-growing number of diseases associated with ion channel function. Channelopathies have been recognized in the context of conditions as diverse as epilepsy, cardiac arrhythmias, skeletal muscle disorders, and diabetes.

Lately, increasing evidence suggests that yet another disease can be ascribed, at least in part, to ion channel malfunction: cancer. Although we are still a long way from cataloguing cancer as a channelopathy, that is, a disorder arising directly from ion channel dysfunction, there is mounting evidence pointing to the involvement of ion channels in cancer progression and pathology. In this review, we will summarize the latest findings concerning the involvement of ion channels in cancer.

The Making of a Cancer Cell

Before considering ion channel involvement in tumor development, a brief discussion of how cancer evolves at the cellular level seems appropriate in order to better understand the context of ion channel participation in this process.

The development of cancer in humans is a multistep process that usually occurs over many decades. This process involves the alteration of genes and/or proteins involved in cell proliferation, apoptosis, and differentiation. Therefore, in the first step, a cancer cell acquires a phenotype that either allows it to proliferate without limits, evade apoptosis, generate its own mitogenic signals or ignore growth-inhibitory ones (see Figure 1).

At a later stage, the cell would need to attract vasculature (angiogenesis) so as to sustain the increasing number of cancer cells. Still later, the cell would need to acquire a phenotype that allows it to invade and colonize (metastasize) neighboring or even distant tissue.

It is generally accepted that the disruption of a relatively small number of genes or proteins with key roles in the above-mentioned pathways is essential for the development of the neoplastic phenotype. Indeed, a mutated version of the ras protein that is constitutively active and therefore signals continuous mitogenic stimuli, has been identified in about a quarter of human tumors. Similarly, the p53 tumor-suppressor protein, which controls DNA repair and apoptosis pathways, is mutated in nearly half of human tumors.

This is not to say that only mutations in these particular proteins can induce neoplastic transformation, but rather that they are key regulators of their specific pathway. For example, overexpression of the tyrosine kinase receptor HER2/neu, which is an upstream regulator of ras, occurs in a large percentage of breast cancers.

While the biochemical pathways involving ras-mediated mitogenic stimulation or p53-directed apoptosis are relatively well understood, the molecular circuitry enabling enhanced secretion of angiogenic factors from cancer cells is largely unknown. Similarly, the regulation of the elements controlling the migration and extravasation capabilities of cancer cells is poorly understood.

The Involvement of Ion Channels in the Neoplastic Phenotype

The contributions of ion channels to the neoplastic phenotype are as diverse as the ion channel families themselves and therefore a comprehensive review of all the channels and their possible functions in cancer progression is beyond the scope of this review. A brief examination of the known (and possible) contribution of ion channels to the biology of the cancer cell will be discussed below.

The largest number of studies are concerned with the involvement of ion channels in cell cycle regulation. Ion channels control cell proliferation in several ways. First, ion channels (mainly voltage-gated K+ channels) control the maintenance of the membrane potential and changes in membrane potential are absolutely required throughout the cell cycle. The other way by which ion channels are involved in cell cycle progression is by controlling cell volume. Cell proliferation must, at some point, lead to cell “swelling”, a closely regulated process that includes activity of K+ and Cl- channels. Not surprisingly, the same ion channel mechanisms that regulate cell proliferation are involved in the control of the other side of the coin: apoptosis. Cell shrinkage is one of the early events marking the onset of apoptotic cell death. Again, a prominent role for K+ and Cl- channels has been established in this process, although the molecular identity of the Cl- channels has not been determined.

Cytosolic Ca2+ increase plays a key role in both proliferation and apoptosis, although the nature of the Ca2+-permeable channels involved and their regulation remains obscure.

The potential role of ion channels in tumor-
induced angiogenesis has not been properly addressed until now. In this process, tumor cells secrete proangiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and others, which stimulate endothelial cells to form blood vessels. Several classes of ion channels are expressed on endothelial cells although their functional role has only begun to be investigated. Invasive growth or cell migration is a highly regulated process in which the migrating cells must secrete matrix proteases that disrupt the extracellular matrix (ECM) and permit easier transit through the surrounding environment. In addition, they must also profoundly reshape their structure, which involves massive cytoskeletal rearrangement. There is increasing information concerning ion channel involvement in cytoskeleton reshaping and cell-cell interaction and also some evidence that tumor cell invasion can be halted by the use of channel blockers (see below). A revision of the accumulated data on the involvement of at least some of the ion channel families in neoplastic transformation will be presented below.

**A. Voltage-Gated K⁺ Channels**

The most compelling evidence concerning the involvement of ion channels in the transformation process is related to the overexpression of voltage-gated K⁺ channels in a variety of cancers. Voltage-gated K⁺ channels is a surprisingly large (about 40 human genes have been identified until now) and ubiquitously expressed protein family. All members of the family consist of a pore-forming unit (also called α subunit) with six transmembrane spanning segments that selectively conduct K⁺ ions across the cell membrane. A typical voltage-gated channel is composed of four α subunits (either homeric or heteromeric) and four (optional) auxiliary β subunits, which have regulatory functions. The voltage-gated K⁺ channel family can be further divided into 4 subfamilies based on amino acid similarity and some functional properties. They are the Kv (Shaker-like), the ether-a-go-go (EAG), the KCNQ and the BK (Ca²⁺-activated K⁺ channels) subfamilies. As their name implies, voltage-gated K⁺ channels open in response to a depolarization of the cell membrane, thus allowing an efflux of K⁺ ions. Channels that belong to the BK subfamily in addition to being voltage-sensitive, can also open in response to an increase in intracellular Ca²⁺. Voltage-gated K⁺ channels are ubiquitously expressed and are the main channels responsible for maintaining membrane potential in diverse cells. There is a strong correlation between membrane potential and cell proliferation: terminally differentiated cells (that do not proliferate) are very hyperpolarized, while cycling cells (such as tumor cells) are very depolarized. It has been suggested that activation of K⁺ channels is necessary for the progression of the cells through the G1 phase of the cell cycle. Indeed, inhibition of these channels by pharmacological agents has been shown to inhibit cell proliferation in both normal activated lymphocytes and various cancer cell lines. For the most part however, the identity of the specific channel involved in cell cycle regulation in the different cell types has not been clearly established. On the other hand, mounting evidence has identified channels of the eag subfamily as highly involved in the development of cancers of both hematopoietic and non-hematopoietic origin. The eag subfamily of voltage-gated K⁺ channels can be subdivided into three distinct groups based on sequence homology. They are the eag, the eag-like K⁺ channels (elk) and the eag-related genes (erg). In the last few years, a growing number of studies have shown that a member of the erg family, the erg1 gene (also known as human erg1 or HERG1), is selectively upregulated in a
variety of human and animal tumors while its expression is absent in the normal tissue or cell line counterparts.\textsuperscript{12-15} (see Figure 2) Moreover, selective pharmacological blockade of the HERG channel in several primary leukemic cells significantly reduced cell proliferation.\textsuperscript{14,15} It is still unclear, however, how over-expression of this particular voltage-dependent $K^+$ channel contributes to the neoplastic phenotype. One possibility is that the special properties of HERG channels contribute to maintain a more depolarized membrane potential and thus permit an easier passage through the cell cycle. In a recent report, it was shown that the oncogene v-src (a constitutively active form of the protein tyrosine kinase src) could phosphorylate the HERG channel and thus induce an increased current.\textsuperscript{16} Since aberrant function of proteins in the ras-src signaling pathway is a common feature of transformed cells as discussed above, src-mediated modulation could be a mechanism that regulates HERG function in cancer cells.

Another study showed that the HERG channel expressed in cell lines and primary tumors was preferentially a heterotetramer formed by the "regular" herg1 gene transcript and an alternative splice variant termed herg1b. The biophysical properties of the resulting channel turned out to be quite different than those exhibited by HERG in normal cells. In addition, the expression of the two HERG protein isoforms was strongly cell cycle-dependent.\textsuperscript{17} Another study demonstrated that HERG protein physically interacted with the tumor necrosis factor receptor type 1 (TNFR1) in the cell membrane of tumor cell lines.\textsuperscript{18} TNFR1 is the ubiquitously expressed receptor for the TNF$\alpha$ cytokine that can mediate both cell proliferation and apoptosis in many cells.\textsuperscript{19} The significance of its interaction with the HERG channel however, is not clear. Another group within the eag-$K^+$ channel subfamily that has been clearly implicated in malignant transformation is the eag group itself. In this group two genes have been identified: eag1 and eag2. The expression of both proteins is largely restricted to the brain, however several groups found evidence indicating that eag1 was inappropriately expressed in several cancer cell lines.\textsuperscript{20-22} Moreover, one study showed that Eag1 by itself had oncogenic potential as a cell line transfected with the channel induced aggressive and faster tumor growth in vivo as compared to a cell line transfected with an unrelated Kv channel.\textsuperscript{22} The same study also showed that inhibition of eag1 expression with antisense oligonucleotides was sufficient to decrease the proliferation of various cancer cell lines. As is the case for the HERG1 protein, the contribution of EAG1 to tumor development is believed to be related to its ability to modulate cell cycle progression.

Another voltage-dependent $K^+$ channel subfamily that can be involved in tumor progression is the BK$_{Ca}$ subfamily. This subfamily is also known as Ca$^{2+}$-dependent $K^+$ channels meaning, as mentioned above, that they allow $K^+$ efflux in response to an increase in intracellular Ca$^{2+}$. BK$_{Ca}$ channel over-expression was identified in primary human gliomas, which showed a positive correlation between BK$_{Ca}$ channel expression levels and tumor malignancy.\textsuperscript{23} Another study showed that specific BK$_{Ca}$ channel blockers could inhibit cell proliferation in an astrocytoma cell line.\textsuperscript{24} Although the number of reports linking BK$_{Ca}$ expression and/or function to cancer development is not large at the moment, it could be worthy to further explore this topic.

As a $K^+$ channel sensitive to intracellular Ca$^{2+}$ levels, BK$_{Ca}$ channels sit at the crossroads of several metabolic pathways including cell proliferation, apoptosis, and cell migration. Indeed, the BK$_{Ca}$ channel has been shown to be a physiological target of the ras protein in fibroblast cell lines. In these cells, BK$_{Ca}$ channel blockers were able to inhibit mitogen-induced cell proliferation indicating that BK$_{Ca}$ is an essential member of the ras-controlled proliferation pathway.\textsuperscript{25}

B. Miscellaneous Ion Channels

An assorted collection of ion channels has been implicated in cancer progression. In most cases, there are only preliminary reports showing an aberrant expression of a particular ion channel in cancer malignancies whereas the biological implications of their abnormal expression are not clear. TRPV6 (also known as CaT1 and CaT-L) is a member of the TRP superfamily of non-voltage-gated cation channels. TRPV6 is a Ca$^{2+}$ selective channel that is believed to be involved in Ca$^{2+}$ reabsorption by epithelial kidney and gut cells. TRPV6 was demonstrated to be abundantly expressed in prostate tumors but not in healthy prostate tissue.\textsuperscript{21} Moreover, its expression was enhanced in a variety of human tumors of epithelial origin.\textsuperscript{22} Another channel that may be involved in tumor progression is P2X7. This channel is an ATP-gated cation channel (permeable to both Ca$^{2+}$ and Na$^+$), which is widely expressed in immune cells. Opening of P2X7 channels by extracellular ATP induces a wide range of biological responses, including cell proliferation, apoptosis, modulation of cytokine secretion, etc. Indeed, it has been shown that P2X7 expression is enhanced in a form of B-cell leukemia.\textsuperscript{31} Finally, the voltage-gated L-type Ca$^{2+}$ channel (Cav1.2 or a1c) was significantly increased in colon cancer epithelial cells when compared with adjacent normal tissue.\textsuperscript{34}
Final Considerations

In the next few years we will probably see an expansion of the increasing list of ion channels implicated in cancer development as the awareness and the tools needed to investigate this issue are more readily available. As is the case with other protein families, it will probably be difficult to ascribe tumor development to the malfunction of a single ion channel. Rather, defects in ion channels probably contribute to the neoplastic phenotype through complex interactions with other proteins, most of which have not been properly identified. Along the same line, there is increasing evidence that ion channel investigation will become an integral part of fields ranging from cell-cell adhesion, arteriosclerosis and immune dysfunction. Since in many cases there are already known pharmacological modulators (blockers and activators) of ion channels, identification of a single defective ion channel in a particular cancer could provide a ready-to-go therapeutic approach.

The opposite can also be true. As the chlorotoxin story beautifully demonstrates, a well-known toxin against a non-identified ion channel can become a potent anti-cancer drug (see below for more details).

In synthesis, in the next few years we are poised to see new and exciting discoveries regarding ion channels and their function in cancer development.

R e f e r e n c e s


I on C hannels in Cancer

The Chlorotoxin Story: From Cl− Channel Inhibitor to Cutting Edge Anti-Cancer Drug.

Chlorotoxin is a 36-amino acid peptide that was originally isolated from the venom of the Leiurus quinquestriatus scorpion as a putative Cl− channel inhibitor.1 It was later found that Chlorotoxin could inhibit invasiveness of glioma cells in vitro. This inhibition was attributed to the ability of Chlorotoxin to block an unidentified Cl− channel that was putatively involved in the process of regulatory volume decrease, a key step in cell migration.2 Interestingly, Chlorotoxin was found to bind specifically to glioma cell lines and primary cultures, but not to normal brain cells. This aspect, together with the proven ability of Chlorotoxin to inhibit glioma cell migration, placed this molecule as an attractive candidate for the treatment of glioma malignancies.3 Indeed, the FDA has recently granted approval for a phase I/II clinical trial using an iodinated Chlorotoxin derivative (125I-Chlorotoxin) for the treatment of brain tumors. Lately, it has been shown that contrary to the original hypothesis, the specific Chlorotoxin target in the surface of glioma cells is the matrix metalloproteinase-2 (MMP-2) protein and not a Cl− channel.4 MMP-2 is a member of a protein family involved in the proteolytic degradation of cell surface and extracellular matrix (ECM) proteins and therefore implicated in the regulation of cell proliferation, differentiation and migration. Metalloproteinases (MPs) have long been recognized as potential targets for the development of anticancer drugs for use in a variety of tumors.5 Until now however, the attempts to develop MP inhibitors have proven ineffective. Chlorotoxin, as a specific MMP2 inhibitor, could then become a useful drug for the treatment of cancer and a range of other diseases. Further research will be needed to establish how binding to MMP2 is connected to the previously reported function of Chlorotoxin as a Cl− channel inhibitor.

We have recently made available, a highly pure, biologically active, recombinant Chlorotoxin (rChlorotoxin; #RTC-450). For more information please refer to the List of Products, table- “Cl− Channel Blocker”.

R e f e r e n c e s

## Ion Channel Modulators

### Technical Information

<table>
<thead>
<tr>
<th>Compound</th>
<th>M.W.</th>
<th>Purity</th>
<th>Specific Channel Modulation Activity and Effective Concentrations</th>
<th>Cat. #</th>
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<tbody>
<tr>
<td>(+)-Bay K 8644</td>
<td>356.3</td>
<td>&gt;99%</td>
<td>Activate L-type; 1µM</td>
<td>B-350</td>
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<tr>
<td>rBeKm-1</td>
<td>4098</td>
<td>&gt;98%</td>
<td>Specifically blocks ERG K+ channel with IC50 of 3.3 nM</td>
<td>RTB-470</td>
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<tr>
<td>Calcicludine</td>
<td>6979</td>
<td>&gt;98%</td>
<td>Neuronal L-type blocker; 1-10nM.</td>
<td>C-650</td>
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<tr>
<td>Calciseptine</td>
<td>7036</td>
<td>&gt;98%</td>
<td>L-type blocker; 100nM - 2µM.</td>
<td>C-500</td>
</tr>
<tr>
<td>rCharybdotoxin</td>
<td>4353</td>
<td>&gt;99%</td>
<td>High (BK) or intermediate (K1) conductance Ca2+-activated and voltage-gated (Kv1.3) blocker; 10 - 100nM.</td>
<td>RTC-325</td>
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<tr>
<td>rChlorotoxin</td>
<td>3996</td>
<td>&gt;99%</td>
<td>Blocks at sub-micromolar concentration small conductance Cl- channel of epithelial cells &amp; also blocks Cl- channels expressed in gliomas.</td>
<td>RTC-450</td>
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<tr>
<td>E-4031</td>
<td>510.48</td>
<td>&gt;98%</td>
<td>Voltage-gated (HERG) blocker; 100 nM - 1µM</td>
<td>E-500</td>
</tr>
<tr>
<td>Ergotoxin-1</td>
<td>4738</td>
<td>&gt;98%</td>
<td>ERG K+ channel blocker; IC50=10nM</td>
<td>RTE-450</td>
</tr>
<tr>
<td>FS-2</td>
<td>7004</td>
<td>&gt;98%</td>
<td>L-type blocker; 100nM - 2µM</td>
<td>F-700</td>
</tr>
<tr>
<td>ribeirotoxin</td>
<td>4231</td>
<td>&gt;99%</td>
<td>High conductance Ca2+-activated K+ channel (BK) blocker; 50 - 100nM.</td>
<td>RTI-400</td>
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<tr>
<td>Isopimaric Acid</td>
<td>302</td>
<td>&gt;99%</td>
<td>BK Channel Opener 1 - 50µM</td>
<td>I-370</td>
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<tr>
<td>rNoxiustoxin</td>
<td>4195</td>
<td>&gt;99%</td>
<td>Some Ca2+-activated and voltage-gated K+ channel blocker; 10 - 100nM.</td>
<td>RTN-340</td>
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<tr>
<td>Pimaric Acid (Plata)</td>
<td>302</td>
<td>85-90%</td>
<td>BK Channel Opener 1 - 50µM</td>
<td>P-270</td>
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<td>TaiCaltoxin</td>
<td>52,000</td>
<td>&gt;97%</td>
<td>L-type blocker; 50 - 500nM</td>
<td>T-800</td>
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### Ordering Information

For price and ordering information please refer to the List of Products.

### Diagrams

**Calcicludine**

The effect of 50nM rBeKm1 on hERG1 channels expressed in Xenopus oocytes. Responses to 200 ms pulses from holding potential of ~100 mV to + 20 mV, before and during application of 50nM rBeKm1, with 2mM K' as charge carrier.

### Legend

- **Control**: Baseline response without the application of the toxin.
- **Calcicludine**: Response after the application of 50nM calcicludine.

**A**: I-V relation before (circle) and during (triangles), bath perfusion of the toxin.

**B**: An example of current response to 200 ms depolarization to +20 mV (from holding potential of ~100 mV) before (red) and during (black) perfusion of the toxin.
The effect of 5µM (±)-Bay K 8644 on heterologously expressed L-type calcium currents (Ca\textsubscript{V}1.2/ α\textsubscript{2}δ\textsubscript{1}/ β\textsubscript{2a}, RNA injected to Xenopus oocytes).

A: I-V relation before (circle) and during (triangles), bath perfusion of the compound.

B: An example of current response to 200 ms depolarization to +20 mV (from holding potential of -100 mV) before (red) and during (black) perfusion of the drug.

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A. hERG channels currents recorded from Xenopus oocytes (in 5mM K\textsuperscript{+}) elicited by 500ms depolarization from holding potential of -100mV to +20mV, before and during application of rErgtoxin-1 at the indicated concentration.

B. Mean ± S.D, dose response for rErgtoxin-1 block of hERG channels (n was between 2-7 oocytes for each point).

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**Related Antibodies**

<table>
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<th>Description</th>
<th>Application</th>
<th>Family</th>
<th>Reactivity Confirmed</th>
<th>Epitope</th>
<th>Epitope location</th>
<th>Cat. #</th>
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<tr>
<td>Anti-Ca\textsubscript{V}1.2\textsubscript{α\textsubscript{1C}} (L-type)</td>
<td>WB, IH</td>
<td>Voltage-Gated \Ca\textsuperscript{2+} Channels</td>
<td>rat, mouse, rabbit, human</td>
<td>Peptide corresponding to residues 848-865 of rat Ca\textsubscript{V}1.2 (α\textsubscript{1C}).</td>
<td>Intracellular loop between II and III domains.</td>
<td>ACC-003</td>
</tr>
<tr>
<td>Anti-BK\textsubscript{α\textsubscript{1C}}</td>
<td>WB, IH</td>
<td>K\textsuperscript{+} Channels</td>
<td>rat, mouse</td>
<td>GST fusion protein corresponding to residues 1098-1196 of mouse Slo (mSlo).</td>
<td>Intracellular, C-terminus.</td>
<td>APC-021</td>
</tr>
<tr>
<td>Anti-erg1</td>
<td>WB, IH</td>
<td>K\textsuperscript{+} Channels</td>
<td>rat, human</td>
<td>Peptide corresponding to residues 1122-1137 of rat erg1.</td>
<td>Intracellular, C-terminus.</td>
<td>APC-016</td>
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<tr>
<td>Anti-HERG</td>
<td>WB, IH</td>
<td>K\textsuperscript{+} Channels</td>
<td>human</td>
<td>GST fusion protein corresponding to residues 1106-1159 of human erg (HERG).</td>
<td>Intracellular, near the C-terminus.</td>
<td>APC-062</td>
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<tr>
<td>Anti-EAG2</td>
<td>WB</td>
<td>K\textsuperscript{+} Channels</td>
<td>rat</td>
<td>Peptide corresponding to residues 842-860 of rat EAG-2.</td>
<td>Intracellular, C-terminal.</td>
<td>APC-053</td>
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<tr>
<td>Anti-P2X7</td>
<td>WB, IH</td>
<td>Purinergic Receptors</td>
<td>rat, mouse</td>
<td>Peptide corresponding to residues 576-595 of rat P2X7.</td>
<td>Intracellular, C-terminus.</td>
<td>APR-004</td>
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For price and ordering information please refer to the List of Products, tables.
Western blotting of rat brain membranes:
1. Anti-α_{1C} antibody (#ACC-003) (1:200).
2. Anti-α_{1C} antibody, preincubated with the control peptide antigen.

Western blotting of the lysate of HEK 293 cells, stably expressing H150G channels (the line generously provided by Dr. Craig T. January, University of Wisconsin):
1. Anti-erg1 antibody (#APC-016) (1:200).
2. Anti-erg1 antibody, preincubated with a control peptide antigen.

Western blotting of rat brain membranes:
1. Anti-P2X7 antibody (#APR-004) (1:1000).
2. Anti-P2X7 antibody, preincubated with a control peptide.

Staining of the interpeduncular nucleus (IPN) with Anti-BK_{Ca} antibody (#APC-021) green fluorescence.
Contributed by Shai Shoham Ph.D.
Herzog Hospital, Jerusalem.

Western blotting of rat brain membranes:
1. Anti-BK_{Ca} antibody (#APC-021) (1:300).
2. Anti-BK_{Ca} antibody, preincubated with the control antigen.