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The use of optical biosensors to detect modulation of Slack potassium channels by G-protein coupled receptors

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Abstract

Ion channels control the electrical properties of neurons and other excitable cell types by selectively allowing ion to flow through the plasma membrane. In order to regulate neuronal excitability, the biophysical properties of ion channels are modified by signaling proteins and molecules, which often bind to the channels themselves to form a heteromeric channel complex. Traditional assays examining the interaction between channels and regulatory proteins generally provide little information on the time course of interactions in living cells. We have now used a novel label-free technology to detect changes in the distribution of mass close to the plasma membrane following modulation of potassium channels by G-protein coupled receptors (GPCRs). This technology uses optical sensors embedded in microplates to detect changes in the refractive index at the surface of cells. Although the activation of GPCRs has been studied with this system, protein-protein interactions due to modulation of ion channels have not yet been characterized. Here we present data that the characteristic pattern of mass distribution following GPCR activation is significantly modified by the presence of a sodium-activated potassium channel, Slack-B, a channel that is known to be potently modulated by activation of these receptors.

Introduction

Ion channels in the nervous system primarily function to generate a wide variety of firing patterns that are required for processing of sensory information and generation of motor outputs. Ion channels control the electrical properties of neurons and other excitable cell types by selectively allowing ions to flow. Most ion channels can exist in different conformational states, an open (conducting) state which allows ions to flow, and a closed (non-conducting state) which prevents ions from flowing [1]. Protein kinases, phosphatases, and other enzymes linked to signaling pathways, as well as ligand binding, modulate the conformational state of ion channels, allowing a neuron to modulate its behavior to external stimuli by triggering a change in the conformational state of the ion channel.

Many of these signaling molecules bind to the ion channel, either directly or through intermediaries, forming heteromultimeric protein complexes [2]. For example, protein kinase A (PKA) has been shown to bind to ion channels through A kinase anchoring proteins (AKAPs) to form regulatory complexes [3]. The signaling molecule calmodulin (CaM) tethers to voltage-gated calcium channels, regulating the conformation state of the channel. Binding of calcium to CaM causes inactivation of the channel, providing a negative feedback loop for calcium entry into the cell [4, 5]. These are merely a few examples of hetromultimeric protein complexes that mediate ion channel activity.

A traditional view of ion channel modulation has been that of a “one way street” in which the properties of the channel are altered by intracellular biochemical events but that the

channel itself does not alter downstream signaling pathways. Recent research has shown, however, that many ion channels can directly influence biochemical signaling mechanisms through processes that are not directly linked their functions in permitting ion flux [6]. Bi-directional control over cellular signaling has been shown in each of the major classes of ion channels that influence excitability, including the sodium, calcium and potassium channels that shape action potentials, as well as the non-selective cation channels that control resting membrane properties [6]. As ion channels often exist in heteromultimeric protein complexes with these signaling molecules, bidirectional signaling may lead to protein-channel or protein-protein interactions in which a protein binds to or is released from the channel complex at the cell membrane. Following a signaling event, such differential binding of proteins would be expected to produce a redistribution of protein mass at locations immediately under the plasma membrane.

Assays examining the interaction between channels and regulatory cytoplasmic proteins, such as co-immunoprecipitation assays, generally provide little information on the time course of interactions in living cells and sometimes require modifications of the proteins of interest, which can potentially alter the protein behavior. A new and promising method for detecting interactions between ion channels and regulatory proteins that is capable of detecting interactions in intact cells, and that does not require the introduction of exogenous labels, is based on the use of optical sensors embedded in microplates to detect changes in the refractive index at the surface of cells (Corning Epic system) [7, 8]. A redistribution of mass close to the plasma membrane (<150 nm from the sensor surface), such as that caused by signaling protein binding or release from membrane bound proteins, causes a change in the index of refraction of light impinging on the microplates. This assay for screening of intact cells has been termed the Mass Redistribution Cell Assay Technology (MRCAT.) This technology uses a resonant waveguide grating biosensor to monitor ligand-induced dynamic mass redistribution (DMR) within the first ~150 nm of the surface of adherent cells [9]. Changes in the index of refraction are detected as a shift in resonant wavelength. DMR may occur as proteins or other large molecules associate with or dissociate from the plasma membrane of a cell. This system, which provides a non-invasive and label-free optical assay to measure the DMR of proteins and signaling molecules upon receptor activation, not only allows the study of cell signaling mechanisms, but also permits high throughput screening of compounds directed against surface receptors.

The changes in mass distribution that results from the conformational change of the ion channel protein associated with opening or closing of the channel pore in the plasma membrane would be expected to be too small to produce a signal detectable by optical sensors. Nevertheless, if channel activation were to be accompanied by changes in the association of the channel protein with other cellular components a significant DMR could be detected. Recent research in our laboratory has also shown that pharmacological activation of one type of ion channel, the Slack-B sodium-activated potassium (K_{Na}) channel, leads to a dynamic mass redistribution. In this paper, however, we shall focus on changes in DMR that are associated with the modulation of such channels by GPCRs [10].

We shall first provide a brief and selective overview of some of the known interactions between ion channels, regulatory proteins and signaling molecules in cellular signaling pathways. While many of these interactions may represent high affinity stable associations between the channel and cytoplasmic components, others may be more labile and, such signaling molecules and regulatory proteins may bind and dissociate upon channel activation [2]. Such association/dissociation of proteins or other molecules with channels would be expected to cause a mass redistribution close to the cell membrane, and may therefore be detected with Epic optical biosensor system [7]. Then we will present evidence for the Slack-B sodium-activated potassium (K_{Na}) channel, which modulates the DMR that occurs

upon G protein-coupled receptor (GPCR) activation. The Slack-B channel has been shown to be strongly modulated by GPCRs [11].

Some examples of ion channel signaling pathway interactions

Channels interact with MAP kinase and CaMKinase II pathways

Expression of the potassium channel Kv10.1, named EAG (for ether-a go-go), occurs primarily in neuronal synaptic endings [12, 13]. These channels have been shown to use their voltage-sensor domain to alter the activity of the MAP kinase signaling pathway [14]. Transfection of the *eag* gene into NIH 3T3 fibroblasts or C2C12 myoblasts has been shown to increase cell density and stimulate DNA synthesis [14]. This effect is mediated by an increase in p38 MAP kinase activity. Although this occurs regardless of the channels ability to conduct ions, mutations that shift the voltage-dependence of activation towards negative potentials, biasing the channel into its open state, block the proliferative effects of the channels. Thus, the EAG channel must be in a closed state in order to activate p38 MAP kinase. Channel conformation can therefore be seen as a switch for the signaling activity of EAG and a link between channel activity and intracellular signaling. The activation of the MAP kinase pathway by EAG may cause signaling molecules to associate or dissociate with the channel complex near the cell surface, thereby causing a dynamic mass redistribution. EAG channels also interact directly with the calmodulin dependent protein kinase CamKII [15].

Calmodulin is a dynamic regulator of ion channels

Calmodulin (CaM) is a calcium sensor protein involved in numerous biochemical signaling pathways. Increased calcium concentrations cause CaM to bind calcium, and the Ca^{2+} -bound CaM can modulate numerous targets, including cellular signaling molecules [16]. The role of CaM in the inactivation of calcium-dependent voltage-gated calcium channels has been well described in the literature [17]. As these channels are permeable to calcium, inactivation of the channels by Ca^{2+} -bound CaM provides negative feedback regulation of calcium entry into the cell by inactivating channels. CaM also functions as the calcium sensor for both small (SK) and intermediate (IK) conductance calcium-activated potassium channels [4, 5]. SK channels are gated solely by calcium, independent of membrane voltage. Because CaM is usually firmly tethered to the channel complex, changes in the activity of CaM due to changes in calcium levels would not be expected to induce binding or dissociation of the CaM protein. Nevertheless, Ca^{2+} -bound CaM does activate numerous targets, including calmodulin-dependent protein kinases (CaM kinases), which may differentially interact with the tethered CaM, providing a potential link between channel activity and redistribution of mass.

Large conductance (BK) potassium channels interact with multiple signaling pathways

The large conductance calcium-activated potassium channel (also termed BK channel,) is regulated in a multicomponent signaling complex. These voltage gated K^+ channels are modulated by calcium binding to an intracellular site or sites on the α -subunit that forms the pore. In mammals, at least four different β -subunits have been identified that modulate the channel. The *Drosophila* BK channel, although lacking the β -subunits, is modulated by numerous partners. c-Src and cAMP-dependent protein kinase (PKA) have been shown to regulate the BK channel by binding to and phosphorylating the channel [18]. Two other modulatory proteins, Slob [19] and Slip [20], also bind to the α -subunit of BK channels. Slob can bind to the signaling protein 14-3-3, and recruit the signaling protein to the channel complex. Differential phosphorylation states of Slob determine binding to 14-3-3, allowing for a dynamic regulation of the proteins constituting the BK channel multi-protein complex

[21]. Such dynamic regulation of BK-associated proteins during cellular signaling could cause a significant redistribution of mass close to the plasma membrane.

Regulators of presenilins and of transcription can associate with potassium channels

Proteins in the KChIP (**K Channel Interacting Proteins**) family are ancillary subunits to the Kv4 family of potassium channels. They also have functions independent of the channel itself. Four proteins have been identified, termed KChIP1-4, which bind directly to the Kv4 α -subunits, and modulate the amplitude and kinetics of inactivation of the channels [22]. Before their role as potassium channel regulators was discovered, however, KChIPs were, found to function as Ca^{2+} -binding proteins that regulate presenilins [23], then as Ca^{2+} -dependent regulators of transcription [24]. Presenilin 1 and 2 are transmembrane protein components of a large, multisubunit aspartyl protease complex that has been named γ -secretase. This complex is required for the intramembranous cleavage of a number of membrane proteins [25]. These two roles of KChIPs provide a possible mechanism to link Kv4 channel activity with changes in transcription and of presenilin-dependent cleavage of membrane proteins, the latter of which is likely to be associated with a significant redistribution of mass at the plasma membrane.

G proteins bind to ion channels

G protein-coupled receptors (GPCRs) are a superfamily of cell membrane proteins that are involved in a vast number of cell signaling mechanisms. Agonist binding to the receptor causes the coupled heterotrimeric protein to dissociate, releasing the α - and $\beta\gamma$ -subunits. Release of these subunits activates numerous downstream targets, allowing for amplification of the signal at the biochemical level. One family of potassium channels, the G protein-gated inwardly rectifying potassium channels (GIRKs), has been shown to be regulated directly by G proteins. Binding of the $\beta\gamma$ heterodimer to the channel triggers channel opening, potassium ion flux, and hyperpolarization of the cell [26]. Another family of ion channels that are regulated by G proteins is that of the voltage-gated calcium channels (VGCCs), which like the GIRKs, are regulated by direct binding of the $\beta\gamma$ heterodimer to the channel [27].

Activation of GPCRs is expected to result in a redistribution of mass at the membrane as the α - and $\beta\gamma$ -subunits either dissociate or associate with the receptor. Previous research has shown that activation of all three classes of G-protein coupled receptors, Gs-, Gq-, and Gi-coupled receptors, cause a DMR signal that is readily detected with the optical biosensor technique [8].

Properties of the sodium-activated potassium (K_{Na}) channels *Slack* and *Slick*

We have given a brief and selective account of channel-protein interactions that could provide dynamic changes in mass distribution following activation of signaling pathways. More comprehensive reviews of this subject are to be found in Kaczmarek (2006) [6] and Levitan (2006) [2]. We shall now describe the properties of a class of channels that have very large cytoplasmic domains, which are potently regulated by GPCRs, and for which changes in DMR using optical biosensors can readily be detected following activation of these receptors and following direct activation of the channels themselves. These are the sodium-activated potassium channels encoded by the *Slack* and *Slick* genes (Fig. 1).

Although the existence of sodium-activated potassium currents has been known for over 20 years [28], the molecular basis of these channels has been identified only relatively recently. Our laboratory has identified the genes for two sodium-activated potassium (K_{Na}) channels, *Slack* and *Slick*, that are highly expressed in the central nervous system [29]. These potassium channels are sometimes described as members of the *Slo* family of potassium

channels, and within the literature, have also been referred to as *Slo2.2* (*Slack*) and *Slo2.1* (*Slick*). Slack (Sequence like a Ca^{2+} -activated K^{+} -channel) and Slick (Sequence like an intermediate conductance K^{+} channel) share only a 7% sequence homology with their nearest homologue, the large conductance (BK) channel (Slo) [30–32]. Slack and Slick do not possess the positively charged S4 domain characteristic of other voltage-sensitive channels. Unlike Slo, the N-termini of Slack and Slick are believed to reside in the cytosol. Slack is the largest K^{+} channel subunit known, and contains two regulators of K^{+} conductance (RCK) domains in its large C terminal domain [33]. Slack also has a consensus PDZ-binding motif at the end of its C-terminal tail, which may be functionally important, since Na^{+} channels and glutamate receptors, which conduct the K_{Na} activator Na^{+} , are also known to cluster by binding to PDZ domains [34].

Slack cDNA has been expressed in mammalian HEK and CHO cells and *Xenopus* oocytes, and the whole cell current of these transfected cells indicates an instantaneous component followed by a slow, time dependent increase in current [30–32]. Inside-out patches of Slack-B channels have a conductance of ~180 pS in symmetrical potassium solution, and sub-conductance states that are detected frequently and range from 30–60 pS. Although Slack does not possess a positively charged S4 domain, it is outwardly-rectifying in both physiological and in symmetrical saline solutions. Cytoplasmic addition of Na^{+} results in a dose dependent increase of Slack-B activity that is reversible upon lowering Na^{+} concentration. Although channel activity is enhanced, unitary conductance remains unchanged, as would be expected for K^{+} selective channels. Slack-B channel activity cannot be detected in the absence of cytoplasmic Na^{+} [32]. Concentration-response curves for activation of Slack-B give an effective concentration of Na^{+} for 50% activation (EC_{50}) of ~41mM, and Slack-B binds Na^{+} cooperatively, with a Hill coefficient of 2.4. Both values are within the range of K_{Na} channels found in native tissues [29].

There are multiple splice isoforms of the Slack channel. The *Slack* gene is encoded by 30 exons, and in humans is located on chromosome 9q34.3. The two that have been most intensely investigated are *Slack-A* and *Slack-B* [35]. The exon encoding the N-terminal of Slack-B is located ~10 kb away from the next exon. Through searching gene databases with the N-terminal of Slick, we found another putative Slack exon 15 kb upstream of the N-terminal of Slack-B. The coding region of this exon is almost identical (>95%) to the N-terminus of Slick. This isoform of Slack was designated Slack-A, and encodes a protein of 1,203 amino acids with the N-terminal nearly identical to that of Slick, and the remainder of the sequence identical to Slack-B. In contrast to Slack-B, current in Slack-A transfected cells activate very rapidly with step changes in voltages, similar to that of Slick whole cell currents, and unlike Slack-B currents which have a slow component.

Native K_{Na} channels in the auditory system have been shown to adapt the pattern of neuronal firing to different rates of stimulation and influence the fidelity of information transmitted [36]. Computer modeling and biophysical and pharmacological experiments has also shown the Slack and Slick channels regulate the accuracy of firing of these neurons. In addition to these functions, the very large cytoplasmic domain of Slack has been shown to interact with a variety of cytoplasmic signaling molecules, making the channel an attractive candidate for determining if activation or modulation of these ion channels can influence the DMR in optical biosensor assays.

Slack and Slick are potently regulated by GPCRs

The activity of both Slack and Slick channels has been shown to be very strongly influenced by activation of $\text{G}\alpha_q$ protein coupled receptors such as the M1 muscarinic receptor and the mGluR1 metabotropic glutamate receptor, as well as by direct activation of protein kinase C (PKC) [11]. Immunocytochemical studies of brain sections and of cultured neurons have

demonstrated co-expression and co-localization of these channel subunits with these GPCRs. Experiments in which the channels were co-expressed with these GPCRs in the *Xenopus* oocyte expression system demonstrated that Slick and Slack channels are modulated in opposite ways. Activation of these receptors by PKC produces a strong reduction of Slick currents. In contrast, activation of the same pathways produces a 2–3 fold increase in the amplitude of Slack currents and significantly slows the rate at which these current activate on depolarization. By construction of chimeric channels containing the N-terminal and membrane spanning domains of Slack with the C-terminal cytoplasmic domain of Slick, it was demonstrated that this differential modulation by GPCRs and PKC reside in the C-terminal domain and the specific phosphorylation sites responsible for this modulation are being characterized [37].

Expression of Slack alters the DMR response that follows GPCR activation

Previous research has shown that upon activation of all three classes of G-protein coupled receptors, Gs-, Gq-, and Gi-coupled receptors, can be detected with the optical biosensor technique (Epic system). To determine whether the DMR produced by activation of GPCRs can be altered by the presence of ion channels that are modulated by receptor activation we have compared the pattern and magnitude of the DMR induced by the muscarinic agonist carbamylcholine chloride (carbachol) in control HEK-293 cells with that in HEK-293 cells expressing the Slack-B potassium channel.

In order to verify that Slack channel subunits were expressed at the plasma membrane in the HEK-293 cells stably expressing the *Slack-B* gene, we first used conventional patch-clamping methods to verify that typical sodium-activated Slack potassium currents could be recorded from these cells. These Slack-B expressing HEK cells do not differ in apparent morphological or growth properties from the non-transfected (control) HEK cells. Both the Slack-B expressing HEK cells and the control HEK cells were plated onto a Corning Epic plate at equal densities and allowed to incubate overnight in order to generate a single monolayer of cells covering the biosensor. Then cells were incubated at 26° C in the Epic system for 2 hours prior to assay measurements. Baseline optical measurements were taken for 4.5 minutes, after which the plate was removed from the measuring system and compounds were rapidly added with an automated liquid handling system. The plate was returned to the machine and measurements were taken for the next 90 minutes.

Figure 2A demonstrates that simply removing either the control or the Slack-expressing cells from the measurement system, washing them with external medium, and then replacing them for further measurements produced no significant change in the DMR measurement. Application of carbachol to control HEK cells resulted in a positive increase in DMR, that has been described previously [38] (Fig 2B). This control response peaked at ~10 minutes and then decayed slowly over the remainder of the 90 minute measuring period (calculated $t_{0.5} = 35.6$ min). In the Slack-B expressing cells, application of carbachol also produced a positive shift in the DMR (Fig 2C). Compared to the control cells, however, the response peaked earlier (at ~5 minutes) and was of a greater magnitude ($p < 0.001$). Decay from the peak response in the Slack-B transfected cells also differed from that in control cells. Rather than a single slow decay, the DMR signal in Slack-B transfected cells first decayed rapidly for ~ 15 minutes from its peak (calculated $t_{0.5} = 17.8$ min), and then decayed slowly at a rate comparable to that in control for the remainder of the 90 minute measuring period (calculated $t_{0.5} = 41.4$ min).

We also compared the concentration-response relations for the action of carbachol on the DMR in control and Slack-expressing HEK cells (Fig. 3A,B). The differences in the kinetics of the response, with the existence of an early rapid peak in the Slack-expressing cells were apparent at all concentrations of carbachol.

We also carried out similar experiments using another agonist of GPCRs in HEK cells. SFLLR, an N-terminal TRAP pentapeptide which exhibits agonist activity against the GPCR endogenous protease activated receptor 1 (PAR1), was also applied at a concentration of 5 μ M. In control untransfected HEK cells, the time course of the DMR response to SFLLR differed from that of carbachol in that the response was more sustained without a significant slow decay over measurement lasting up to 90 minutes. As with carbachol, however, expression of Slack-B altered the kinetics and amplitude of DMR upon activation of this GPCR (Fig. 4). A significant increase in the DMR occurred in Slack-B transfected cells, as compared with control cells ($p < 0.0001$).

In summary, the presence of the Slack-B channel significantly alters the DMR that occurs upon activation of endogenous GPCRs in HEK cells. With Slack-B expressing cells the observed DMR is larger in amplitude and differs in its kinetic behavior from that observed in untransfected cells.

Conclusions

For many years ion channels were considered to be plasma membrane components that have relatively few interactions with cytoplasmic signaling pathways. It is now understood, however, that many and perhaps all ion channels exist as multi-protein complexes with activities that are highly integrated with a wide variety of cytoplasmic and nuclear signaling events [2, 6]. We are still, however, at an early stage of understanding how these channel complexes form and how they are regulated. Here we have presented several select examples of ion channels that have interactions with regulatory proteins and molecules, and potential mechanisms by which bidirectional cellular messaging may occur. This binding and release of signaling proteins and molecules to and from ion channel complexes would be expected to cause a dynamic mass redistribution in regions very close to the plasma membrane.

We have also demonstrated that over-expression of the Slack-B sodium-activated potassium channel in HEK cells that express endogenous GPCRs produces a very significant change in the pattern of mass redistribution close to the plasma membrane following activation of these receptors. Use of an optical biosensor (Corning Epic) allowed us to monitor the time course and magnitude of these DMR changes without the introduction of exogenous labels into either the ion channel itself or into endogenous components of GPCR signaling pathways. The molecular mechanism of the change in DMR is not yet known. One plausible mechanism by which this may occur is that Slack-B forms a complex either with the endogenous GPCRs or with downstream signaling components, such as protein kinase C, which is known to be a key regulator of Slack currents [11]. The formation of multi-protein complexes with Slack could potentially be mediated the PDZ domain at the C-terminus of the Slack protein [39]. Another possibility is that the observed DMR responses reflect cytoskeletal changes following channel modulation. We cannot also eliminate the possibility that the alterations in DMR reflect rapid dynamic alterations in the adherence of the cells to the substrate.

Future experiments will be required to identify the proteins or signaling molecules that contribute to the alterations of the DMR following modulation of Slack-B or of other channels. One possibility will be to attempt to eliminate components of the DMR response using RNAi against proteins that are believed to interact with the channel or with other proteins of the channel complex. Ongoing research has indicated that very significant changes in DMR can also be produced simply by agents that activate Slack-B channels directly, without activation of GPCRs [10]. Such findings suggest that, in addition to probing molecular mechanisms of channel protein-protein interactions, optical biosensor

may be used in high throughput screening of novel compounds that influence channel activity and lead to the discovery of pharmacological activators and inhibitors in a very much shorter time-frame than that of traditional multi-channel electrophysiological screening methods.

Materials and Methods

Reagents

Carbamoylcholine chloride (Carbachol) was purchased from Sigma Chemical Inc., St. Louis, MO. SFLLR peptide was purchased from Ana Spec, San Jose, CA. Corning Epic 384 well biosensor microplates, coated with Fibronectin, were purchased from Corning, Inc., Corning, NY.

Cell Culture

Human embryonic kidney 293 (HEK-293) cells were purchased from American Type Cell Culture. HEK-293 cells stably expressing rat Slack-B were generously donated by Yangyang Yan in the laboratory of Dr. Fred Sigworth at Yale University. For cell culturing, cells were grown in one half Dulbecco's Modified Eagle medium (Gibco #11965) and one half Leibovitz's L-15 Medium (Gibco #11415) supplemented with 10% heat inactivated fetal bovine serum and antibiotics. 300 µg/mL Geneticin (Gibco #11811-031) was added to HEK-293 cells stably expressing Slack-B to select for expression of the Slack channel. Cells were plated on the Corning Epic biosensor plates at 12,000 cells per well and incubated overnight at 37° C under air/5% CO₂ to provide a single monolayer of cells covering the biosensor. Cells were washed 5x with Hanks Balanced Salt Solution, with calcium chloride and magnesium chloride (HBSS, Gibco #14025), with a Cy-Bi® Well 384 Well automated liquid handling system. Before assay measurements, cells plated to the Corning Epic biosensor plates were incubated in the Epic system for 2 hours at 26° C.

Dynamic Mass Redistribution (DMR) Optical Biosensor Assays

The Epic plates were removed from incubation and cells were washed 5x with 1x HBSS by a Cy-Bi Well 384 Well automated liquid handling system and allowed to incubate at 26° C in the Epic system for 2 hours prior to assay measurements. Details of the instrumental setup and assay protocols have been previously described (Fang, Ferrie, Fontaine, & Yuen, 2005.) All compound solutions were prepared in HBSS and added to cells with a Cy-Bi® Well 384 Well automated liquid handling system.

To calculate the rate of change of the DMR signal in untransfected HEK cells over time (Figures 2 and 3), we calculated a half time of decay ($t_{0.5}$) using a single exponential fit ($N(t) = N_0 2^{-t/t_{0.5}}$) to all of the data points after the peak response to agonist had occurred. For Slack-B transfected cells, in which two decay rates could clearly be differentiated, single exponential fits, using the same equation were calculated separately for the first 15 min following the peak agonist response, and for the decay that occurred subsequent to this 15 min period.

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Figure 1.

Principal domains of Slack and Slick channels and summary of characteristics of these channels at the macroscopic and single channel level. A) Slack has two isoforms, Slack-A and Slack-B, with the C-terminal of both isoforms being nearly identical. Slack B-has a much longer N-terminus than Slack-A, which shares 95% sequence homology with the N-terminus of Slick. B) The pharmacology and kinetics of Slack-B and Slick. Whole cell currents (upper inserts) were recorded in response to 200 ms pulses between -120 and +120 mV. Single channel data (bottom inserts) were recorded at a membrane potential of +80 mV. Modified from Bhattacharjee and Kaczmarek, 2005 [29].

Figure 2.

Expression of Slack-B alters the DMR response to activation of muscarinic receptors by carbachol. A) Slack-B transfected or control HEK-293 cells were treated with HBSS buffer alone, which did not cause a significant response. B) Untransfected HEK cells and C) Slack-B transfected HEK-cells were exposed to 75 μ M carbachol, and a highly statistically significant difference in the amplitude of DMR observed was detected ($p < 0.001$, $n = 6$). A slow decay in signal was observed in untransfected HEK cells ($t_{0.5} = 36.6$ minutes), whereas Slack-B transfected cells had two decay rates ($t_{0.5} = 17.8$ min, 41.4 min; see Methods for details of calculation).

Figure 3.

The DMR response to carbachol in control and Slack-B expressing cells is dose-dependent. A) Increasing the concentration of carbachol applied to cells increases the DMR observed for untransfected and Slack-B transfected cells. B) Increasing carbachol concentrations has a greater effect on the DMR of Slack-B transfected than control HEK cells.

Figure 4.

Expression of Slack-B alters the DMR response following activation of the PAR1 GPCR. Application of SFLLR (5 μ M), an N-terminal TRAP pentapeptide that exhibits agonist activity against the GPCR endogenous protease activated receptor 1 (PAR1), produced a DMR that is greater in amplitude in Slack-B transfected cells than in control cells ($p < 0.0001$, $n = 24$), and differs in its time-course in the Slack-B expressing cells.