Aberrant Subcellular Dynamics of Sigma-1 Receptor Mutants Underlying Neuromuscular Diseases

Adrian Y. C. Wong, Elitza Hristova, Nina Ahlskog, Louis-Alexandre Tasse, Johnny K. Ngsee, Prakash Chudalayandi, and Richard Bergeron

Neuroscience, Ottawa Hospital Research Institute, Ottawa (A.Y.C.W., E.H., N.A., L.-A.T., J.K.N., P.C., R.B.), and Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa (J.K.N., R.B.), Ontario, Canada

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ABSTRACT

The sigma-1 receptor (σ-1R) is an endoplasmic reticulum resident chaperone protein involved in a plethora of cellular functions, and whose disruption has been implicated in a wide range of diseases. Genetic analysis has revealed two σ-1R mutants involved in neuromuscular disorders. A point mutation (E102Q) in the ligand-binding domain results in the juvenile form of amyotrophic lateral sclerosis (ALS16), and a 20 amino-acid deletion (Δ31–50) in the putative cytosolic domain leads to a form of distal hereditary motor neuropathy. We investigated the localization and functional properties of these mutants in cell lines using confocal imaging and electrophysiology. The σ-1R mutants exhibited a significant increase in mobility, aberrant localization, and enhanced block of the inwardly rectifying K⁺ channel Kᵢ,2.1, compared with the wild-type σ-1R. Thus, these σ-1R mutants have different functional properties that could contribute to their disease phenotypes.

Introduction

The sigma-1 receptor (σ-1R) is an endoplasmic reticulum (ER) chaperone protein expressed in neuronal and non-neuronal cells. The σ-1R is widely distributed throughout both the peripheral and central nervous system (Gundlach et al., 1986; Largert et al., 1986; Zuzin et al., 1986; Walker et al., 1992) and is enriched in lower motor neuron cell bodies of the spinal cord (Mavlyutov et al., 2010). As such, the σ-1R has been implicated in diseases associated with motor neuron dysfunction, such as amyotrophic lateral sclerosis (ALS) (Rothstein, 2009; Nassif et al., 2010; Al-Saif et al., 2011; Matus et al., 2013) and a group of genetically and clinically heterogeneous diseases known as distal hereditary motor neuropathies (dHMN) (Rossor et al., 2012; Li et al., 2015).

The σ-1R is a highly evolutionarily conserved, 223 amino acid mammalian protein consisting of a single transmembrane domain (Schmidt et al., 2016). A single missense mutation in the second exon of the SIGMAR1 gene is associated with a juvenile form of ALS (ALS16; Al-Saif et al., 2011). This mutation results in a substitution of glutamine for glutamic acid at position 102 (E102Q) located in the linker region between β2 and β3, which form the base of the ligand-binding β-barrel (Schmidt et al., 2016). Another SIGMAR1 gene mutation (c151+1G>T) has been identified in a consanguineous Chinese family (Li et al., 2015). This mutation leads to an in-frame deletion of 60 base pairs in exon one, generating a σ-1R that lacks the α2 helix encoded by amino acids 31–50 (Schmidt et al., 2016). Expression of this shortened σ-1R variant results in a dHMN phenotype characterized by significant muscle atrophy (Li et al., 2015).

Previous studies using confocal and electron microscopy techniques have demonstrated that the σ-1R is an ER-resident protein, which is highly concentrated at mitochondria-associated membranes (MAM) (Hayashi and Su, 2009, 2007a; Mavlyutov and Ruoho, 2007; Mavlyutov et al., 2012, 2015a). In addition, the σ-1R is present on nuclear envelopes and enriched at lipid rafts (Hayashi and Su, 2003a,b) where it regulates many cellular functions (Su et al., 2010; Kourrich et al., 2012; Nguyen et al., 2015; Su, 2015). In the absence of agonist, the σ-1R is bound to binding immunoglobulin protein (BiP) Hayashi and Fujimoto, 2015; Su, 2015). Upon ligand activation, the σ-1R dissociates from BiP and can modulate the activity of a number of ion channels in the ER (Hayashi and Su, 2007a) and at the plasma membrane (PM) (Aydar et al., 2002; Zhang and Cuevas, 2002; Tcheder et al.,...
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TABLE 1

Primer sequences and strategy for assaying the α-1R-Δ31-50 construct

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>SigmaE102QF</td>
<td>5'-CCTCGCTGTCCTCAGTACGTTG-3'</td>
</tr>
<tr>
<td>SigmaE102QR</td>
<td>5'-CCTGACGAATAACACATGGCGCCG-3'</td>
</tr>
<tr>
<td>Sig1ExF</td>
<td>5'-GACCGCCCAAGAAGCCCGG-3'</td>
</tr>
<tr>
<td>Sig1intdelR</td>
<td>5'-CCTGGTTGTGGCTGGGGCTGGACCATGAGCTTGCCTTC-3'</td>
</tr>
<tr>
<td>Sig1intdelF</td>
<td>5'-CACCAGCTGTCAGTGTCCTTCCTTC-3'</td>
</tr>
<tr>
<td>Sig1Int seq</td>
<td>5'-GCTGACATGGTTGATTTGGTA-3'</td>
</tr>
<tr>
<td>Sig1R seq</td>
<td>5'-ACTTCGTCGTTGGCCCTCTT-3'</td>
</tr>
</tbody>
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Materials and Methods

Plasmids and Constructs. The α-1R-YFP cDNA was cloned into the pcDNA3.1/His cloning vector (Invitrogen, Carlsbad, CA) as previously described elsewhere (Hayashi and Su, 2003a). This plasmid was used as a template for the generation of the other α-1R constructs. The α-1R-E102Q-YFP construct was generated by standard site directed mutagenesis using Phusion High Fidelity Polymerase (Finnzyme; New England BioLabs, Ipswich, MA) using the phosphorylated primer pair SigmaE102Q F and SigmaE102Q R indicated in Table 1, according to manufacturer's instructions. This was followed by DpnI digestion to remove traces of the initial template DNA, and column purification. The plasmid was then used to transform chemically competent NEB 5a E. coli (New England BioLabs). Positive clones were screened by sequencing using the primer Sig1Fseq. Confirmed clones were further verified by sequencing the entire α-1R gene with primers Sig1Fseq and Sig1Rseq.

A fusion polymerase chain reaction (PCR) approach was used to generate α-1R-Δ31-50-YFP (Table 1). The α-1R-YFP was subcloned into pCDNA3.1 as an EcoRI-BamHI fragment. For the first PCR, primer pair Sig1ExF and Sig1intdel R were used in a 100-μL reaction with Phusion GC buffer supplemented with 3% dimethylsulfoxide. The second PCR was performed with the same template DNA but using the primer pair Sig1intdel F and Sig1Ex R. Each of the PCR products was digested with DpnI to remove traces of the initial template DNA then column purified. A 5-μl aliquot of each of the clean PCR products was mixed together in a separate tube and diluted 100-fold. This served as the template for the third PCR using the primer pairs Sig1Ex F and Sig1Ex R. The PCR product was then digested with EcoRI and BamHI, and the ∼680 base-pair band was gel isolated. We also digested α-1R-YFP with EcoRI and BamHI, and the ∼5 kilobase vector was gel isolated. The vector and insert were ligated together, and the resulting construct was transformed into NEB5a cells. The colonies were screened for the α-1R-Δ31-50 insert, and the entire α-1R was sequenced to verify the deletion of amino acids 31–50. To generate the α-1R-mCherry tagged protein, the α-1R sequence was subcloned as an EcoRI-BamHI fragment into the pLVX-mCherry-N1 vector (Clontech Laboratories, Madison, WI) resulting in mCherry (mCh) tag at the C-terminal end of the receptor.

The pQC NLS mCherry IX (NLS-mCh) plasmid was a gift from Connie Cepko (Beier et al., 2011) (Addgene plasmid 37354), and the humanstromal interaction molecule-1 (STIM1)-YFP plasmid was a gift from Anjana Rao (Prakriya et al., 2006) (Addgene plasmid 19754). The BiP-mCh was a gift from Erik Snapp (Lai et al., 2010) (Addgene plasmid 62233). The Kir2.1 plasmid was a gift from Dr. Jean-Claude Béguè (Mizuno et al., 2007).

Cell Culture. Mouse embryonic fibroblasts (MEF) were isolated from C57/BL6 mouse embryos (E14.5) as previously described elsewhere (Xu, 2005). In brief, E14.5 fetuses were isolated, and the visible organs were removed. The tissue was treated with 0.25% trypsin-EDTA and triturated. The dissociated cell suspension was cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum, 100 U/ml penicillin/streptomycin, and 1× GlutaMAX (all from Life Technologies-Thermo Fisher Scientific, Burlington, Canada). The isolated MEFs were grown in a humidified 37°C, 5% CO2 incubator and passaged every 3–4 days using trypsinization. For all experiments, the cells were plated at a density of ~0.2 × 106 cells/ml on 15-mm Thermanox plastic coverslips (Thermo Fisher Scientific) in 12-well plates. The next day, the cells were transiently transfected using TransIT-2020 transfection reagent (Mirus, Madison, WI) according to manufacturer’s instructions and imaged 48–72 hours after transfection. For electrophysiologic recordings, MEFs were transiently transfected with α-1R-YFP, α-1R-E102Q-YFP, or α-1R-Δ31-50-YFP, and Kir2.1 with mCh as a marker for transfected cells at a ratio of 5:1 and recorded from 24 hours after transfection.

Chinese hamster ovary (CHO) cells were maintained in Dulbecco's modified Eagle medium/Ham's F-12 medium (1:1) with 10% fetal bovine serum, 100 U/ml penicillin/streptomycin, and 1× GlutaMAX in a similar manner as described earlier for MEFs. Transfection was also performed according to the same protocol.

Live-Cell Imaging. We performed live-cell imaging for transfected MEFs and CHO cells using confocal lasers at 488, 515, and 559 nm on an upright Olympus BX61WI (Olympus America, Center Valley, PA) with a 60 × (NA 1.0) water immersion objective. The plasma membrane was stained with CellMask Deep Red plasma membrane dye.
stain (Life Technologies/Thermo Fisher Scientific) as a morphologic marker 20 minutes before imaging. During the experiments, the cells were continuously bathed in an external solution containing (mM): 150 NaCl, 10 HEPES, 3 KCl, 1 MgSO\(_4\), 2 CaCl\(_2\), 10 Na-ascorbate, pH 7.4, with a flow rate of ~1 ml/min.

For the morphologic analysis, the cells were imaged at a resolution of 1024 \(\times\) 1024 pixels, dwell time of 4 \(\mu\)s/pixel. Z-stacks were obtained with a 0.75–1 µm step size (6–10 sections per stack).

For fluorescence recovery after photobleaching (FRAP) experiments, a circular region of interest was outlined and photobleached using 100% laser power for 500 milliseconds after 5 frames of prebleach baseline. Cells were imaged at 256 \(\times\) 256 resolution, 0.5 milliseconds pixel dwell time, for 2 seconds/frame, every 10 seconds for 300 seconds (5 minutes) for the short FRAP experiments or every 30 seconds for 1200 seconds (20 minutes) for longer duration experiments.

**Western Blot Analysis.** For all Western blot samples, an equal volume (10 µl) of sample was resolved on 10% SDS-PAGE, transferred onto polyvinylidene fluoride membrane and developed using Luminata Crescendo (Millipore, Darmstadt, Germany). The bands were detected using film, and subabsorbed bands were used for quantification of the pixel intensities using ImageJ (National Institutes of Health) (Schindelin et al., 2012). Western blot experiments were repeated 3 times from different sets of transfected cells using three plates per set, and the band intensities were normalized to \(\beta\)-actin before comparison. We used \(\beta\)-actin as a loading control.

**Antibodies.** The following antibodies and dilutions were used in this study: rabbit polyclonal anti–green fluorescent protein (1:1000; Novus Biologicals, Oakville, Canada), goat polyclonal anti–\(\alpha\)-1R (1:250; Santa Cruz Biotechnology, Dallas, TX), and mouse monoclonal anti–\(\beta\)-actin (1:14,000; GenScript, Piscataway, NJ). The horseradish peroxidase–conjugated secondary antibodies were all purchased from Jackson ImmunoResearch (West Grove, PA).

**Electrophysiology.** Whole-cell voltage-clamp recordings were performed on MEFs transiently transfected with K\(_{\text{v}}\)2.1 and mCh together with the YFP-tagged \(\alpha\)-1R or \(\alpha\)-1R mutants. Cells were imaged under epifluorescence or confocal microscopy, and only those that had both YFP and mCh fluorescence were selected for recordings. Thick-walled borosilicate glass electrodes (1.5 mm o.d., 0.9 mm i.d.) were filled with a K\(^+\)-glutamate solution containing (mM): 115 K\(^{+}\)-Glu, 20 KCl, 10 HEPES, 4 Mg\(^{2+}\)-ATP, 0.5 GTP, 10 Na\(^{+}\)–phosphocreatine. The pH was adjusted to 7.4 using 5 N KOH, and osmolarity was set to 290 mOsm using sucrose. The \(E\) \(_{K}\) was calculated to be ~80 mV using the Nernst equation. MEFs were held at a membrane potential of 0 mV and hyperpolarized to membrane voltages between ~170 and ~30 mV for a 1-second duration (30-second interstimulus interval). The step protocol was repeated every 5 minutes, and the recordings were stable for up to 1 hour using this protocol. Drugs were dissolved directly into the external solution and bath applied to the cell.

**Drugs.** We obtained (25,65,11S)-1,2,3,4,5,6-hexahydropyridine-6,11-dimethyl-3-(2-propenyl)-2,6-methano-3-benzazocin-8-ol hydrochloride (SKF-10,047; SKF) and N-(2-(3,4-dichlorophenyl)ethyl)-N-methyl-2-(dimethylamino)ethylamine dihydrobromide (BDI1047; BD) from Tocris (Ellisville, MO), and (+)-pentazocine (PTZ) from Sigma-Aldrich (Dorset, United Kingdom). All other chemicals and salts were obtained from Sigma-Aldrich (Oakville, Canada).

**Analysis.** Confocal image stacks were analyzed using the Fiji/ImageJ (Schindelin et al., 2012) colocalization plugin Coloc2 (http://fiji.sc/Coloc2) using the automated Manders method (Manders et al., 1993) with a calculated point spread function and 10 Costes shuffling iterations (Costes et al., 2004). Electrophysiologic data were analyzed using Clampfit (Molecular Devices, Sunnyvale, CA). Whole-cell currents were leak subtracted before analysis; the peak amplitude was the largest negative-going amplitude in the first 50 milliseconds of the step, and the steady-state current was the mean of the last 100 milliseconds of the step.

Data were graphed using Microcal Origin 8.0 (Malvern Instruments, Worcestershire, United Kingdom) and expressed as mean \pm S.E.M. Significance was determined using a two tailed Student’s t test, with \(P < 0.05\) considered statistically significant.

**Results**

**Wild-Type \(\sigma\)-1R Is Localized to the ER.** Previous studies using confocal imaging (Hayashi and Su, 2003a,b; Mavlyutov and Ruoho, 2007; Mavlyutov et al., 2012) and electron microscopy (Mavlyutov et al., 2015a) have demonstrated that the \(\sigma\)-1R is an ER-resident protein that appears to concentrate at the ER-mitochondrion interface (Hayashi and Su, 2007a) and at lipid droplets (Hayashi and Su, 2003b; Hayashi and Su, 2004). Moreover, subsequent work has demonstrated that the \(\sigma\)-1R binds to binding immuno-globulin protein (BiP) when not activated by an agonist (Hayashi and Su, 2007a). However, the subcellular dynamics of the two \(\sigma\)-1R mutants that underlie neuromuscular diseases, \(\sigma\)-1R-E102Q (Tagashira et al., 2014) and \(\sigma\)-1R-A31–50 (Li et al., 2015), are poorly characterized. We performed localization experiments using confocal imaging on primary MEF or CHO cells transiently transfected with the fluorescently tagged proteins \(\sigma\)-1R-YFP and BiP-mCh to determine their intracellular localization. Previous work has demonstrated that the YFP-tagged \(\sigma\)-1R behaves identically to the endogenous, untagged \(\sigma\)-1R (Hayashi and Su, 2003a). Thus, we will use the designation WT \(\sigma\)-1R to refer to the \(\sigma\)-1R-YFP construct. Likewise, the YFP tagged \(\sigma\)-1R mutants under investigation (\(\sigma\)-1R-E102Q-YFP and \(\sigma\)-1R-A31–50–YFP) will be designated \(\sigma\)-1R-E102Q and \(\sigma\)-1R-A31–50 respectively.

Z-stack projections revealed a reticular pattern for both the \(\sigma\)-1R and BiP with extensive colocalization between the two (Fig. 1A). The reticular distribution of \(\sigma\)-1R-YFP was recapitulated using a \(\sigma\)-1R-mCh construct that was cotransfected with a green fluorescent protein–tagged STIM1. STIM1 is a sensor of Ca\(^{2+}\) levels in the ER and forms part of the store operated calcium entry response after Ca\(^{2+}\) depletion from the ER (Kraft, 2015; Moccia et al., 2015). As expected, STIM1-YFP overlapped extensively with \(\sigma\)-1R-mCh (\(\sigma\)-1R-mCh; Fig. 1B). There was no significant overlap with the CellMask Deep Red plasma membrane stain (CM; Fig. 1). To investigate the possible nuclear localization of the \(\sigma\)-1R (Miki et al., 2013), \(\sigma\)-1R-YFP was cotransfected with NLS-mCh (Beier et al., 2011) (Fig. 1C). No colocalization was observed between \(\sigma\)-1R-YFP and NLS-mCh, suggesting extranuclear or perinuclear \(\sigma\)-1R-YFP localization.

Colocalization analysis revealed that there was a high degree of colocalization between \(\sigma\)-1R-YFP and BiP-mCh (Pearson’s coefficient [PC] = 0.54 \pm 0.07; \(n > 25\) cells from 5 different coverslips), and between STIM1-YFP and \(\sigma\)-1R-mCh (PC = 0.79 \pm 0.05; \(n > 8\); Fig. 1D). As expected, there was minimal colocalization between \(\sigma\)-1R-YFP and NLS-mCh (PC = 0.11 \pm 0.04; Fig. 1D).

In addition, we performed an intensity correlation analysis (Li et al., 2004). This analysis generates an intensity correlation quotient (ICQ), where an ICQ of 0.5 represents perfect colocalization, 0 no colocalization and –0.5 perfect inverse correlation (Li et al., 2004). The ICQ was 0.32 \pm 0.07 for \(\sigma\)-1R-YFP and BiP-mCh, and 0.41 \pm 0.09 for STIM1-YFP and \(\sigma\)-1R-mCh (\(n = 5\) coverslips, \(\geq 5\) stacks per coverslip), indicating a high degree of colocalization (Fig. 1E). In contrast, the ICQ between \(\sigma\)-1R-YFP and NLS-mCh was 0.04 \pm 0.005 (\(n = 5\)), demonstrating a lack of colocalization between these two proteins.

Taken together, these results indicate that the \(\sigma\)-1R is localized to the reticular ER, in agreement with previously published work (Hayashi and Su, 2003a,b, 2007a,b; Mavlyutov and Ruoho, 2007;
Mavlyutov et al., 2012, 2015a). These data are the basis by which the subcellular distribution of the α-1R mutants can be compared.

Reticular and Punctate Distribution of α-1R-E102Q. We next examined the distribution of the two α-1R mutants. Expression of α-1R-E102Q-YFP in primary MEFs revealed a strikingly different expression pattern to that observed with the WT α-1R. In contrast to the reticular pattern of the WT α-1R, the E102Q mutant accumulated in large puncta, which was accompanied by a concomitant decrease in reticular signal (Fig. 2A). No apparent plasma membrane localization was observed (Fig. 2, A and B). The localization of BiP-mCh was unaffected by the presence of α-1R-E102Q (Fig. 2A). Interestingly, the distribution of NLS-mCh was affected by α-1R-E102Q, resulting in diffuse, putatively cytoplasmic staining in addition to nuclear staining (Fig. 2B). As the α-1R is able to translocate into the nucleus (Miki et al., 2013), this suggests that expression of α-1R-E102Q mutant may alter nucleocytoplasmic shuttling or affect the nuclear retention of NLS-mCh.

Quantification of the images revealed a significant decrease in colocalization of α-1R-E102Q with BiP (PC = 0.36 ± 0.06; P < 0.05, n = 34 cells from >3 coverslips; Fig. 2D) compared with that of WT α-1R (0.54 ± 0.07), which is apparent in the

![Image](https://example.com/image_url)
higher magnification image (Fig. 2C). However, there was a significant increase in colocalization between \( \sigma-1R\)-E102Q and NLS-mCh (PC = 0.30 ± 0.07; \( P < 0.05 \), \( n = 3 \); Fig. 2D) due to the resulting diffuse cytoplasmic distribution of NLS-mCh. Intensity correlation analysis resulted in an ICQ of 0.17 ± 0.06 between \( \sigma-1R\)-E102Q and BiP-mCh, and 0.12 ± 0.04 between \( \sigma-1R\)-E102Q and NLS-mCh, both of which are significantly different from \( \sigma-1R\)-YFP (\( P < 0.05 \), \( n = 3 \) coverslips Fig. 2E).

In addition to the decrease in colocalization with BiP, \( \sigma-1R\)-E102Q formed large puncta. Measurement of punctal size revealed that \( \sigma-1R\)-E102Q puncta were approximately 3 times larger than those observed for \( \sigma-1R\)-YFP (\( \sigma-1R\)-E102Q: 293 ± 25 nm; \( \sigma-1R\)-YFP: 105 ± 7.3 nm; \( P < 0.05 \), \( n = 3 \); Fig. 2, F–H).

Whether these structures are due to aggregation of mutant \( \sigma-1R\) or segregation into a distinct subcompartment of the ER remains to be determined.

**Diffuse Cellular Distribution of \( \sigma-1R\)-D31–50.** Transient transfection of the \( \sigma-1R\)-D31–50-YFP mutant into either MEFs or CHO cells revealed a diffuse and nonreticular staining pattern, while the distribution patterns of BiP-mCh, NLS-mCh, and CM were unchanged (Fig. 3, A and B). This distribution is reminiscent of a soluble protein, which
was unexpected as the transmembrane domain remains intact and amino acid residues 31–50 are not part of a predicted ER retention sequence (Brune et al., 2013; Schmidt et al., 2016).

One possible explanation for the apparent soluble distribution of the α-1RΔ31–50-YFP mutant is that the YFP tag is cleaved off and the free YFP remains in the cytoplasm. To examine this, we performed Western blot experiments on MEFs transfected with the α-1R-YFP or either one of the two mutant constructs, probing for free as well as bound YFP. The percentage of free YFP was calculated by dividing the intensity of the free YFP band (Fig. 3C) by the total intensity of bound and free YFP (Fig. 3D). There was significantly lower

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**Fig. 3.** Standard deviation Z-stack projection of MEFs transiently transfected with α-1RΔ31–50-YFP (green) and (A) BiP-mCh (red) or (B) NLS-mCh (red) and counterstained with CM (white). There is diffuse expression of α-1RΔ31–50-YFP while the distribution pattern of BiP-mCh and NLS-mCh remains unaffected. (C, D) Western blot showing the extent of YFP cleavage in MEFs transfected with the α-1R constructs. Lane 1 is α-1R YFP, lane 2 is α-1R-E102Q-YFP, and lane 3 is α-1RΔ31–50-YFP probed with an antibody against YFP. β-Actin is shown as a loading control. The percentage of free YFP was calculated by dividing the intensity of the free YFP band with the sum of both the bound and free YFP bands. (E, F) Western blot showing differential localization of α-1R-YFP and α-1RΔ31–50-YFP in crude cytosolic (E, left) or membrane (E, right) fractions. Endogenous α-1R is used as a marker for membrane fraction and β-actin is shown as a loading control. Pooled data are mean ± S.E.M. of three experiments. Scale bars: 20 μm. Asterisks indicate statistical significance between α-1R-YFP and α-1RΔ31–50-YFP groups (unpaired Student’s t test; *P < 0.05).
cleavage of σ-1R-Δ31–50-YFP (7.67% ± 1.83%) compared with σ-1R-YFP (25.1% ± 5.14%; P < 0.05, n = 3). The amount of YFP cleavage of σ-1R-E102Q-YFP was similar to that for σ-1R-YFP (19.1% ± 7.82%; P = 0.69, n = 3). These data demonstrate that the cytosolic distribution of σ-1R-Δ31–50-YFP is not an artifact of YFP cleavage.

To confirm that the intact σ-1R-Δ31–50-YFP construct is indeed cytoplasmic, we obtained whole-cell lysates from MEFs transfected with σ-1R-YFP or σ-1R-Δ31–50-YFP and then subjected to a 100,000 relative centrifugal force, 90-minute centrifugation to separate the cytosolic from membrane proteins. Western blot experiments were performed on both the supernatant (cytosolic proteins) and the pellet (membrane proteins). Both fractions were probed for the bound YFP–protein. Both fractions were probed for the bound YFP–protein. The percentage of cytosolic σ-1R-YFP or σ-1R-Δ31–50-YFP was calculated by dividing the intensity of the cytosolic band by the sum of the total intensity of cytosolic and membrane-associated bands (Fig. 3E). Quantification of the data shows that 68% ± 8.0% of σ-1R-YFP is associated with membranes, with the remainder being cytosolic (Fig. 3P). In contrast, 67% ± 9.1% of the σ-1R-Δ31–50-YFP mutant was cytosolic, with 31% ± 12% associated with membranes (P < 0.05, n = 3). The endogenous σ-1R was only detected in the membrane protein fraction (Fig. 3E), suggesting that the cytosolic localization of σ-1R-YFP may be an overexpression artifact.

Taken together, these data reveal that both the σ-1R mutants displayed distinct subcellular distribution patterns when compared with σ-1R-YFP or with each other. Live-cell confocal experiments showed that the σ-1R-E102Q-YFP mutant had a reduced reticular distribution and formed large puncta. In contrast, σ-1R-Δ31–50-YFP was evenly distributed throughout the entire cell, with no evidence of puncta. Subsequent biochemical experiments demonstrated that the σ-1R-Δ31–50-YFP is predominantly cytoplasmic.

**Redistribution of σ-1R-YFP after Agonist Application.** Agonist administration results in the dissociation of the σ-1R from BiP (Hayashi and Su, 2007b), leading to increased interaction of the σ-1R with a large variety of ER and PM proteins (Su et al., 2010; Kourrich et al., 2012). This suggests that agonist activation of the σ-1R could lead to an increase in its mobility and possible transport to other intracellular compartments. We tested this hypothesis by imaging cells transfected with σ-1R-YFP and BiP-mCh in the absence of (Fig. 4A) and 60 minutes after (Fig. 4B) the administration of the selective σ-1R agonist SKF 10,047 (SKF, 100 μM) into the culture medium. Standard Manders colocalization analysis (Manders et al., 1993) revealed no significant change in colocalization between σ-1R and BiP in the presence of SKF (Manders coefficient [MC] = 0.62 ± 0.17 in control and 0.33 ± 0.11 in SKF; P = 0.09, n = 10; Fig. 4C). However, a significant increase in punctal size was observed after SKF treatment (control: 97 ± 17 nm, SKF: 178 ± 55 nm; P < 0.05, n = 15; Fig. 4D). This suggests that activated σ-1R tends to cluster in large membrane structures.

These experiments were repeated on cells transfected with σ-1R-E102Q-YFP to investigate the possibility that σ-1R-E102Q could be constitutively active as the mutant receptor formed large puncta under basal condition. If it were the case, addition of SKF should not affect the distribution pattern or puncta size of σ-1R-E102Q. Indeed, there was no change in σ-1R-E102Q and BiP colocalization in the presence of SKF (control: MC = 0.39 ± 0.08, SKF: MC = 0.35 ± 0.1; P = 0.71, n = 10; Fig. 4E and F). Likewise, there was no change in the size of σ-1R-E102Q puncta in the presence of SKF (control: 272 ± 30 nm, SKF: 259 ± 19 nm; P = 0.86, n = 10; Fig. 4G).

**Mobility of Reticular σ-1R-YFP Is Increased after Agonist Application.** Our previous data had revealed that there is a redistribution of WT σ-1R during agonist application, suggesting an increase in σ-1R mobility in the presence of the agonist as the σ-1R moves to form punctal aggregates. To test this, we performed FRAP experiments in the presence and absence of the σ-1R agonists PTZ or SKF.

Figure 5, A and B, shows representative time-lapse confocal images from a FRAP experiment performed on a MEF expressing σ-1R-YFP (Fig. 5A) and BiP-mCh (Fig. 5B) in the absence of agonist. The bleached region of interest is indicated by a white circle placed in the reticular ER, and images were taken every 30 seconds for 20 minutes. At 30 seconds after bleaching, there was nearly full recovery of BiP-mCh fluorescence, while there was significantly less recovery of σ-1R-YFP fluorescence even after 1000 seconds (BiP: 87% ± 2.8%, σ-1R: 47% ± 5.4% of prebleach level; P < 0.05, n = 5; Fig. 5C). This indicates that recovery of BiP was rapid and did not involve changes in ER architecture.

On the other hand, WT σ-1R has low mobility in the ligand-free state with a mobile fraction (f_m) of 0.32 ± 0.07 compared with 0.89 ± 0.05 for BiP. The diffusion constant for the WT σ-1R was best fitted with a double exponential function with a weighted mean of 170 ± 24 seconds, significantly slower than observed for BiP (34 ± 7.2 seconds; P < 0.05, n = 5). Bath application of either of the σ-1R agonists significantly increased the recovery of reticular σ-1R with respect to control (PTZ: 70% ± 4.7% of prebleach level, f_m = 0.64 ± 0.13; SKF: 71% ± 7.3% of prebleach level, f_m = 0.68 ± 0.13; P < 0.05, n = 5; Fig. 5D). Taken together, our FRAP data indicate that the σ-1R becomes more mobile in the presence of the classic agonists SKF and PTZ.

To verify that this change in mobility is due to σ-1R activation, we repeated the experiment after a 20-minute pretreatment the selective σ-1R antagonist BD (Matsumoto et al., 1995). There was no effect of 10 μM BD alone on the recovery of σ-1R-YFP (control: 49% ± 11%; BD: 47% ± 7.4%; P = 0.65, n = 3; Fig. 5E). Likewise, there was no effect of BD on recovery kinetics or mobile fraction (τ = 148 ± 18 seconds, f_m = 0.28 ± 0.08). The increase in σ-1R mobility observed with PTZ was abolished the presence of BD (49% ± 10% recovery, f_m = 0.26 ± 0.09; P = 0.47, n = 3; Fig. 5E). Furthermore, there was no significant change in the mobility of BiP in the presence of PTZ control 87% ± 2.8%; SKF: 85% ± 2.6%; P = 0.33, n = 5). This suggests that the increase in σ-1R mobility is specific to the σ-1R and not simply due to changes in ER dynamics or architecture in the presence of PTZ.

**Mobility of σ-1R Mutants Is Unaffected by Agonist Treatment.** We next examined the mobility of the σ-1R mutants in the absence and presence of SKF. Figure 6, A and B, shows representative FRAP images taken from a MEF overexpressing σ-1R-E102Q-YFP (Fig. 6A) or σ-1R-Δ31–50-YFP (Fig. 6B). The mobility of reticular σ-1R-E102Q-YFP was significantly greater than σ-1R-YFP under baseline conditions (σ-1R-E102Q-YFP: 79% ± 9.2%; σ-1R-YFP 47% ± 5.4%; P < 0.05, n = 4; Fig. 6C). No further increase in mobility was observed in the presence of SKF (SKF: 96% ± 10%; P = 0.09, n = 4; Fig. 6C). Because σ-1R-E102Q also formed large
cytoplasmic puncta, we photobleached and monitored the fluorescence recovery of these structures. As shown in Fig. 5D, these s1R-E102Q puncta exhibited less mobility than reticular s1R-E102Q (40% ± 6.8% for puncta; compare 79% ± 9.2% for reticular s1R-E102Q-YFP). Moreover, its mobility was unaffected by application of SKF (SKF: 45% ± 5.2%; P=0.51, n=4; Fig. 6D). Thus, s1R-E102Q is distributed into at least two distinct membrane compartments. The s1R-E102Q localized to reticular ER is highly mobile, while s1R-E102Q clustered in large puncta is relatively immobile.

The s1R-Δ31–50-YFP mutant has a diffuse distribution throughout the cell, consistent with a soluble cytosolic protein. To further confirm this observation, we performed FRAP experiments using a shortened FRAP protocol, imaging every 10 seconds due to the fast recovery of s1R-Δ31–50-YFP (Fig. 6B). In baseline conditions, s1R-Δ31–50-YFP showed significantly more recovery than s1R-YFP (s1R-Δ31–50-YFP: 47% ± 5.4%; s1R-Δ31–50-YFP: 91% ± 2.5%; P<0.05, n=5; Fig. 6E). However, no significant effect of SKF was observed on s1R-Δ31–50-YFP mobility (SKF: 88% ± 2%; P=0.44, n=5).

In summary, the FRAP data reveal a significant increase in mobility of reticular s1R-YFP in the presence of s1R agonists. In contrast, s1R-E102Q showed markedly increased mobility in baseline conditions, which was not affected by agonist application; this is suggestive of a constitutively active protein. On the other hand, the high baseline mobility, together with the lack of effect of SKF, is consistent with the s1R-Δ31–50-YFP mutant being a soluble cytosolic protein.

Activation of s1R-YFP Results in Modest Blockade of Kᵥ2.1. Ensemble neuronal activity recorded from ALS patients (Vucic et al., 2007; de Carvalho et al., 2012), mouse models (e.g., SOD1-G93A; van Zundert et al., 2012), or single unit recordings from cultured motor neurons (Kuo et al., 2004; Le Masson et al., 2014) reveal chronic and persistent hyperexcitability in motor neurons in ALS. In addition, the s1R KO/SOD1-G93A mouse shows enhanced neuronal activity when compared with the SOD1-G93A mouse alone (Mavlyutov et al., 2015b), suggesting that the s1R may modulate neuronal excitability. Because activation of the s1R leads to the inhibition of voltage-gated K⁺ channels at the PM (Aydar et al., 2002; Renaudo et al., 2004; Zhang and Cuevas, 2005; Martina et al., 2007; Kinoshita et al., 2012; Kourrich et al., 2013), we speculated that this increase in excitability could be mediated via s1R modulation of Kᵥ2.1, a K⁺ channel that is responsible for setting the resting membrane potential in excitable cells (de Boer et al., 2010).

The Kᵥ2.1 subunit was transiently cotransfected into MEFs along with s1R-YFP in equimolar amounts. In addition, mCherry (10-fold lower amount) was transfected as a marker. Only MEFs that expressed both s1R-YFP and mCh were selected for recordings. A sustained, weakly inactivating current was elicited using 1-second duration hyperpolarizing steps from −170 mV to −10 mV using increments of +10 mV from a holding potential of 0 mV (Fig. 7A, top). The interstep interval was 30 seconds, and the protocol was repeated every 5 minutes. Kᵥ2.1 currents were stable for up to 60 minutes.
using this protocol. All currents were normalized to the peak current at −150 mV in control conditions unless otherwise stated. The \( K_{\text{ir}2.1} \) currents had an activation threshold of −76 ± 2.6 mV, in close agreement with the \( E_K \) calculated from the Nernst equation (−79 mV). The current amplitude increased linearly with more hyperpolarized membrane voltages (Fig. 7B). Using the last 50 milliseconds of the current step as a measure of steady-state current, we observed that \( K_{\text{ir}2.1} \) showed minimal voltage-dependent inactivation (−150 mV: 87% ± 3.2% of peak; −100 mV: 93% ± 5.5% of peak; \( P = 0.12, n = 5 \) cells; Fig. 7B), as previously described elsewhere (Kubo et al., 1993). There was no statistically significant difference between MEFs expressing \( K_{\text{ir}2.1} \) alone or in conjunction with \( \alpha\text{-1R-YFP} \), indicating that overexpression of the \( \alpha\text{-1R} \) has little effect on channel properties.

Bath application of SKF (100 μM) resulted in a ∼20% decrease in current amplitude recorded 20 minutes after SKF application (−150 mV: 80% ± 2.3% of control; \( P < 0.05, n = 5 \); Fig. 7B and D). The \( K_{\text{ir}2.1} \) currents were inhibited at all membrane potentials that were more hyperpolarized than the activation threshold in the presence of SKF with no significant change in the activation threshold (SKF: −75 ± 2.3 mV; control: −76 ± 2.6 mV; \( P = 0.33, n = 5 \); Fig. 7B). A nonsignificant decrease in \( K_{\text{ir}2.1} \) inactivation was also observed in the presence of SKF at −170 mV (SKF: 69% ± 1.2%; control: 61% ± 1.8% of peak; \( P = 0.19, n = 5 \)). The inhibitory effect of SKF on \( K_{\text{ir}2.1} \) was reversible, and the current returned to 95% ± 1.6% of control 30 minutes after SKF washout (Fig. 7, B and D).

The effect of SKF on \( K_{\text{ir}2.1} \) is likely due to the activation of \( \alpha\text{-1R} \), as it was abolished after a 20-minute preincubation with BD (BD + SKF: 91% ± 5.5% of control at −150 mV; \( P = 0.69, n = 5 \); Fig. 7, C and D). There was no statistically significant effect on \( K_{\text{ir}2.1} \) current in the presence of BD alone (96% ± 4.1% of control; \( P = 0.72, n = 5 \)). These data indicate that activation of \( \alpha\text{-1R} \) leads to a significant inhibition of \( K_{\text{ir}2.1} \).

**\( \alpha\text{-1R} \) Mutants Significantly Inhibit \( K_{\text{ir}2.1} \) in the Presence and Absence of Agonist.** The FRAP data in Fig. 5 show that the \( \alpha\text{-1R-E102Q} \) mutant is more mobile than the WT \( \alpha\text{-1R} \), which implies that the mutant might be...
constitutively active. Hence, it would be expected to modulate Kir2.1 in an agonist-independent fashion. To test this, we cotransfected MEFs with $\sigma$-1R-E102Q-YFP in addition to Kir2.1 and mCh. The Kir2.1 currents induced in the presence of $\sigma$-1R-E102Q-YFP showed significant voltage-dependent inactivation, even in the absence of agonist, consistent with a constitutively active $\sigma$-1R (Fig. 8A). The steady-state current at $-150$ mV was $63\% \pm 2.31\%$ of the peak in the presence of $\sigma$-1R-E102Q-YFP compared with $87\% \pm 1.3\%$ of the peak in the presence of $\sigma$-1R-YFP ($P < 0.05$, $n = 5$; Fig. 8B). Furthermore, there was a significant decrease in the magnitude of inactivation during steps to successively more depolarized voltages (asterisks in Fig. 8B).

Administration of SKF also resulted in a change in the shape of the IV curve, from linear in control conditions to biphasic, with the break-point at $-110$ mV (Fig. 8C, WT curve in gray). As a result, there was a decrease in peak amplitude at all membrane potentials more hyperpolarized than $-110$ mV ($71\% \pm 1.7\%$ of control at $-170$ mV; $P < 0.05$, $n = 4$; Fig. 8C), but no significant effect of SKF at membrane potentials more depolarized than $-110$ mV. The effect of SKF on peak amplitude was partially reversible and recovered to $80\% \pm 0.6\%$ of control levels 30 minutes after SKF washout. However, the effect of SKF on channel inactivation was not reversed upon washout as the steady-state amplitude was $72\% \pm 1.1\%$ of the peak amplitude at $-150$ mV even after a 30-minute SKF washout. Addition of BD alone had no significant effect on the amplitude of Kir2.1 (96\% ± 3.2\% of control at $-150$ mV; $P = 0.81$, $n = 5$; Fig. 8D), but the effect of SKF was abolished when SKF was administered in the presence of BD (92\% ± 2.1\%, $P = 0.62$, compared with BD alone, $n = 5$; Fig. 8D).

These data show that $\sigma$-1R-E102Q inhibits Kir2.1 in the absence of agonist; however, this inhibition is enhanced in the presence of SKF and is abolished in the presence of BD1047. Taken together, this suggests that $\sigma$-1R-E102Q is partially active under baseline conditions and that the ligand-binding domain is functionally intact in this mutation.

We next examined the effects of $\sigma$-1R-$\Delta$31–50. MEFs transfected with Kir2.1 and $\sigma$-1R-$\Delta$31–50-YFP showed the same
voltage dependence, threshold of activation, and inactivation profile as those transfected with Kir2.1 and α-1R-YFP (Fig. 9, A and B). However, bath application of SKF profoundly reduced the current amplitude at membrane potentials more hyperpolarized than −110 mV (Fig. 9B). Significantly more inhibition was observed at hyperpolarized potentials. For example at −170 mV, SKF blocked 87% ± 6.8% of the current ($P < 0.05, n = 4$) while there was only 30% ± 3.4% block...
at \(-110\) mV \((P < 0.05, n = 4)\). This effect of SKF was not reversible upon washout (Fig. 9B). Interestingly, bath application of BD alone resulted in a significant inhibition of \(K_{\alpha} 2.1\) current at \(-150\) mV \((78\% \pm 6.1\%, P < 0.05, n = 5;\) Fig. 9C); however, there was no additional effect of SKF in the presence of BD \((66.7\% \pm 10.7\%, P = 0.39, n = 4;\) Fig. 9C).

Taken together, these electrophysiologic data show that both \(\sigma-1R\) mutants have drastically different effects on \(K_{\alpha} 2.1\) than the WT \(\sigma-1R\). Activation of \(\sigma-1R\)-E102Q results in a significant inhibition of Kir2.1 current at \(-150\) mV \((78\% \pm 6.1\%, P < 0.05, n = 5;\) Fig. 9C); however, there was no additional effect of SKF in the presence of BD \((66.7\% \pm 10.7\%, P = 0.39, n = 4;\) Fig. 9C).

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**Discussion**

Our data clearly demonstrate that the \(\sigma-1R\) mutants that underlie neuromuscular diseases, \(\sigma-1R\)-E102Q (Al-Saif et al., 2011) and \(\sigma-1R\)-D31–50 (Li et al., 2015), have significantly altered subcellular distribution and mobility when compared with the WT \(\sigma-1R\). As a consequence, these \(\sigma-1R\) mutants have different functional properties that could contribute to their respective disease phenotypes. We found that the mobility of the WT \(\sigma-1R\) is increased after agonist application and leads to modest inhibition of Kir2.1. In contrast, \(\sigma-1R\)-E102Q is highly mobile in the absence of agonist, which suggests that it is constitutively active. Consistent with this is modulation of \(K_{\alpha} 2.1\) by \(\sigma-1R\)-E102Q in the absence of agonist. Finally, the \(\sigma-1R\)-D31–50 mutant behaves as a soluble, cytosolic protein and profoundly inhibits \(K_{\alpha} 2.1\) in the presence of \(\sigma-1R\) agonist SKF.

Although our experiments were performed on cell lines that express the endogenous \(\sigma-1R\), the fact that a robust phenotype is observed in cells expressing the \(\sigma-1R\) mutants suggests that the contribution of the endogenous \(\sigma-1R\) is minimal. This is consistent with studies using tsA201 and MDA-MB-231 cell lines transiently transfected with a tagged \(\sigma-1R\) (Balasuriya et al., 2012).

**Aberrant Subcellular Dynamics of \(\sigma-1R\)-E102Q.** One striking feature of the localization pattern of \(\sigma-1R\)-E102Q was the presence of large, bright puncta, which colocalized poorly with the ER-resident protein BiP. Although little is known about the oligomerization dynamics of \(\sigma-1R\)-E102Q, previous work has suggested that these puncta reflect cytosolic aggregates (Tagashira et al., 2014). Our data also suggest that these aggregates may reflect mutant \(\sigma-1R\) clustering at ER subcompartments where BiP is excluded. One such specialization is the ER quality control compartment (ERQC), which forms during ER stress (Kamhi-Nesher et al., 2001; Frenkel et al., 2004; Kondratyev et al., 2007; Leitman et al., 2013). Indeed, pharmacologic induction of ER stress results in the clustering of the unfolded protein response proteins (UPR) protein kinase RNA-like ER kinase (Kondratyev et al., 2007) and inositol-requiring protein 1 (IRE1) at ERQCs (Kimata et al., 2007; Aragon et al., 2009; Li et al., 2010). In addition, \(\sigma-1R\) clusters are up-regulated by the UPR via the protein kinase RNA-like ER kinase pathway as a protective mechanism after ER stress induction (Mitsuda et al., 2011; Miki et al., 2013). Therefore, the increased aggregation of \(\sigma-1R\)-E102Q could lead to a persistent ER stress condition (Fukunaga et al., 2015), enhancing \(\sigma-1R\)-E102Q clustering at ERQCs.
Aberrant Subcellular Dynamics of α-1R-Δ31–50. Deletion of amino acids 31–50 in α-1R-Δ31–50 resulted in a diffused distribution pattern throughout the entire cell with high mobility resembling a soluble cytosolic protein. This finding was unexpected given that the crystal structure of the α-1R has residues 31–50 assembling in a cytosolic, predominantly α-helical domain (Schmidt et al., 2016). This is on the opposite side of the ER membrane to the presumed double arginine ER-retention motif (Schütze et al., 1994; Hanner et al., 1996). Although it is beyond the scope of this study, we speculate that deletion of amino acids 31–50 could induce gross structural changes that affect α-1R assembly or translocation to the ER, resulting in retention in the cytosol.

Functional Interaction between Kᵦ₂.1 and the α-1R. Kᵦ₂.1 homotetramers form an inwardly-rectifying K⁺ channel (Yang et al., 1995; Collins et al., 1997; Hibino et al., 2010) that is widely expressed throughout the central nervous system (Bredt et al., 1995; Horio et al., 1996; Howe et al., 2008), as well as in cardiac, smooth, and striated myocytes (de Boer et al., 2010; Hibino et al., 2010). Genetic knockout of Kᵦ₂.1 in mice results in death 8–12 hours after birth (Zaritsky et al., 2000); conversely, overexpression of Kᵦ₂.1 leads to a 10-mV hyperpolarization in neuronal resting membrane potential and suppressed neuronal firing (Burrone et al., 2002; Béïque et al., 2011). There is a large body of evidence showing that the α-1R can modulate K⁺ channels at the plasma membrane (Kennedy

Fig. 10. A schematic model to summarize our findings. (A–C) Left panels show cartoons of WT and α-1R mutants, illustrating (B) the destabilized α2±α3 helical domains in α-1R-E102Q (left) and (C) the lack of the α2 helix in α-1R-Δ31–50 (left). Right panels show that activation of α-1R-YFP (A) by SKF (red) leads to an increase in mobility (thicker red arrows) and modest modulation of Kir2.1 (representative traces), potentially via a direct interaction between the two proteins as previously described for other voltage-gated K⁺ channels. (B) The baseline mobility of α-1R-E102Q in the ER is elevated (thicker arrows), and punctal aggregates of putative α-1R-E102Q oligomers are also present. There is enhanced modulation of Kᵦ₂.1 compared with WT α-1R-YFP (red). (C) α-1R-Δ31–50-YFP is excluded from the ER and exists as a highly mobile cytosolic protein that can drastically inhibit Kᵦ₂.1 after SKF administration (red).
and Henderson, 1990; Wilke et al., 1999a,b; Aydar et al., 2002; Zhang and Cuevas, 2005; Kinoshita et al., 2012; Kourrich et al., 2013). In the vast majority of cases, the σ1-IR interacts directly with the ion channel and forms part of the channel complex (Balasuriya et al., 2012). To date, the domains responsible for interaction between the σ1-IR and PM ion channels have not been identified, partially due to ambiguities regarding the localization of the σ1-IR relative to the ion channel. Previous work has suggested a PM localization of the σ1-IR (Aydar et al., 2002; Hayashi and Su, 2007a; Balasuriya et al., 2014; Balasuriya et al., 2013), while more recent electron microscopy experiments in the retina reveal that the σ1-IR is clustered into ER cisternae that are tightly juxtaposed to the PM (Mavlyutov et al., 2015a). Our data appear to support the latter hypothesis as no clear evidence of PM localization of the σ1-IR was observed.

Further studies are needed to fully elucidate the interaction between σ1-IR and voltage-gated ion channels, but activation of the σ1-IR consistently inhibits voltage-gated K⁺ channels (Aydar et al., 2002; Kinoshita et al., 2012; Kourrich et al., 2013; Balasuriya et al., 2014). In agreement with this, we show that activation of WT σ1-IR also inhibits Kᵥ2.1 (Fig. 7). However, this effect is relatively modest, suggesting that the σ1-IR plays only a minor role in regulating neuronal excitability. Furthermore, this implies that the increase in neuronal excitability observed in the σ1-1KO/SOD1-G93A mouse (Mavlyutov et al., 2015b) is likely due to σ1-IR modulation of other K⁺ conductances.

**Functional Interaction between Kᵥ2.1 and the σ1-IR Mutants.** In contrast, the σ1-IR mutants have striking effects on Kᵥ2.1. The voltage-dependent inactivation of Kᵥ2.1 in the presence of σ1-1E102Q is qualitatively similar to that observed in the presence of the classic Kᵥ2.1 pore-blocker barium (Kubo et al., 1993; Liu et al., 2001), suggesting that σ1-1E102Q may interact with the channel pore. Interestingly, examination of the inactivation kinetics of Kᵥ2.1 after activation of σ1-1YFP by SKF reveals a modest increase in voltage-dependent current inactivation, much like what is observed in the presence of σ1-1E102Q. Therefore, σ1-1E102Q appears to behave much like an agonist-activated σ1-IR with respect to its effect on Kᵥ2.1. The mechanism by which this occurs is unclear as crystallographic data show that E102 is not directly with the ion channel and forms part of the channel complex (Burrone et al., 2009, 2013). In the vast majority of cases, the σ1-IR interacts directly with GluN1 but not GluN2A in the GluN1/GluN2A NMDA receptor (Burrone et al., 2009, 2013). The σ1-1 receptor interacts directly with GluN1 but not GluN2A in the GluN1/GluN2A NMDA receptor. J Neurochem. 103:1829–1839.


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**Address correspondence to:** Dr. Richard Bergeron, Neuroscience, Ottawa Hospital Research Institute, 451 Smyth Road, Roger Guindon Hall, Room 3501, Ottawa, Ontario, Canada, K1H 8M5. E-mail: rbergeron@ohri.ca