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SIGMA-1 RECEPTOR EXPRESSION IN THE DORSAL ROOT GANGLION: REEXAMINATION USING A HIGHLY SPECIFIC ANTIBODY

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Abstract—Sigma-1 receptor (S1R) is a unique pluripotent modulator of living systems and has been reported to be associated with a number of neurological diseases including pathological pain. Intrathecal administration of S1R antagonists attenuates the pain behavior of rodents in both inflammatory and neuropathic pain models. However, the S1R localization in the spinal cord shows a selective ventral horn motor neuron distribution, suggesting the high likelihood of S1R in the dorsal root ganglion (DRG) mediating the pain relief by intrathecally administered drugs. Since primary afferents are the major component in the pain pathway, we examined the mouse and rat DRGs for the presence of the S1R. At both mRNA and protein levels, quantitative RT-PCR (qRT-PCR) and Western confirmed that the DRG contains greater S1R expression in comparison to spinal cord, cortex, or lung but less than liver. Using a custom-made highly specific antibody, we demonstrated the presence of a strong S1R immuno-fluorescence in all rat and mouse DRG neurons co-localizing with the Neuron-Specific Enolase (NSE) marker, but not in neural processes or GFAP-positive glial satellite cells. In addition, S1R was absent in afferent terminals in the skin and in the dorsal horn of the spinal cord. Using immuno-electron microscopy, we showed that S1R is detected in the nuclear envelope and endoplasmic reticulum (ER) of DRG cells. In contrast to other cells, S1R is also located directly at the plasma membrane of the DRG neurons. The presence of S1R in the nuclear envelope of all DRG neurons suggests an exciting potential role of S1R as a regulator of neuronal nuclear activities and/or gene expression, which may provide insight toward new molecular targets for modulating nociception at the level of primary afferent neurons.

Key words: Sigma-1 receptor, S1R, dorsal root ganglion, DRG, pain.

INTRODUCTION

The Sigma-1 receptor (S1R), originally proposed as a subtype of the opioid receptor based on benzomorphan opiate binding (Martin et al., 1976), is now known to be a distinct non-opioid receptor protein of 223 amino acids. Its structure was recently solved to be a single transmembrane protein (Schmidt et al., 2016), in contrast to the earlier prediction of a two transmembrane domain protein. The protein primary sequence is unique in the mammalian genome, showing closest similarity to the yeast sterol isomerase (Moebius et al., 1997).

S1R is localized in significant amounts in the endoplasmic reticulum (ER) and is also found in the ER associated mitochondrial membrane (Hayashi and Su, 2007), in the plasma membrane (Kourrich et al., 2013), and more recently is reported to be present in the nuclear envelope (Tsai et al., 2015b).

S1R endogenous ligands include steroids, the trace amine dimethyltryptamine (DMT) (Fontanilla et al., 2009), the lipid sphingosine (Ramachandran et al., 2009), and myristic acid (Tsai et al., 2015a). S1R also exhibits high-affinity binding to exogenous hallucinogenic ligands such as cocaine, ketamine, and haloperidol, suggesting a potential involvement of this receptor in drug addiction. The exact role of S1R in an organism has yet to be elucidated. However, accumulating evidence of pleiotropic modulation of many targets in the plasma membrane, cytosol, and nuclear envelope has led to the suggestion that the S1R is a "pluripotent modulator" of the cell (Su et al., 2016).

In the central nervous system, the S1R protein is expressed in the granular layer of the olfactory bulb, central gray zone, motor nuclei of the hind brain, and in various hypothalamic nuclei (Alonso et al., 2000). At the spinal cord level, a careful comparison between wildtype and S1R-KO mice using a custom-made well-characterized antibody demonstrated a strong immunoreactive (IR) signal in the ventral motoneurons (Mavlyutov et al.,

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Abbreviations: AAV, adeno-associated virus; DRG, dorsal root ganglion; ER, endoplasmic reticulum; IR, immunoreactive; NF200, neurofilament 200; NSE, Neuron-Specific Enolase; PB, phosphate buffer; PFA, paraformaldehyde; qRT-PCR, quantitative RT-PCR; RIPA, radioimmunoassay precipitation buffer; S1R, Sigma-1 receptor; TBD, tetanus binding domain.

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2010). Consistent with the broad expression of S1R in the central nervous system, many reports have suggested an association of this receptor with a variety of nervous system diseases including amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and depression (Su et al., 2016). Another well-described neurologic phenotype in which S1R may have a potential role is inflammatory and neuropathic pain, an association which is well supported by genetic and pharmacological manipulations (Cendán et al., 2005; Kim et al., 2006; Entrena et al., 2009; Gris et al., 2015). The likely role of S1R in pain is also suggested by an unbiased expression screening of genes regulated by the sciatic nerve axotomy model of neuropathic pain, which demonstrated a 2- to 5-fold increase in the expression of S1R in the dorsal root ganglion (DRG) (Xiao et al., 2002). In fact, the S1R is emerging as a novel target in the therapeutic intervention for pain (Zamanillo et al., 2013; Davis, 2015; Gris et al., 2015).

Although the exact role of S1R in pathological pain remains unknown, its abundance in the DRG is interesting. The DRG is the anatomical location that houses the cell soma of the primary afferent sensory neurons and is a critical organ for nociceptive signal processing. The DRG has also been an emerging target for intervention since its location in the peripheral nervous system allows more accessibility relative to the other sites in the central nervous system (Sapunar et al., 2012). A recent report indicated abundant expression of the S1R in the DRG (Bangaru et al., 2013). However, we sought to reexamine the cellular and subcellular anatomical distribution of S1R in the DRG of both rats and mice using a highly-specific well-characterized S1R-antibody (Ramachandran et al., 2007).

EXPERIMENTAL PROCEDURES

Animals

All studies were approved (Protocol M02512, and M02569) by the local institutional animal care use committee, and all animals were treated in accordance with published NIH standards. Male Sprague–Dawley rats weighing 300 g were purchased from Envigo Lab (Envigo, Madison, WI, USA). Oprs1 mutant (+/−) B6;129S5-*Sigmar1*$^{Gt(OST422756)Lex}$/Mmucd mouse litters on a C57BL/6J × 129s/SvEv mixed background were purchased from the Mutant Mouse Regional Resource Center (#011750, MMRRC, UC Davis, CA, USA). All mice and rats were maintained on a normal 12-h light/dark cycle and handled in accordance with animal care and use guidelines of the University of Wisconsin, Madison. Animals were maintained on a 4% fat diet (Harkland Teklad, Madison, WI, USA) with food and water available *ad libitum*.

Two of two-month-old male rats, two S1R wildtype and two S1R knockout mice were used for histological evaluation with confocal and immunoelectron microscopy. For confocal microscopy a minimum of four sections were stained with each antibody. For immunoelectron microscopy a minimum of dozen ultrathin sections were examined per animal.

Intrathecal injections

Mice were deeply anesthetized by isoflurane and 10 μl of AAV2/8-eGFP (5.6×10^{13} vg/ml) (University of Iowa Viral Vector Core, Iowa City, IA, USA) were delivered to mice between L4 and L5 spinal segments intrathecally using a 30-gauge needle. The injection was administered by gripping gently the rodent iliac crest and inserting the needle at a 45° angle in the central point between hip bones. For analysis of GFP expression in the spinal cord and DRG, animals were euthanatized and intracardially perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB) 4 weeks after injection.

Intraplantar and tongue injections

For intraplantar injections, under 2% isoflurane anesthesia, a Hamilton syringe was inserted into the plantar surface of the paw and 1 μl of 1 μg/μl of Cy3 fluorescently-conjugated receptor-binding domain of tetanus toxin was injected. The Cy3-tetanus binding domain (TBD) was prepared in a stapling reaction essentially as described (Darios et al., 2010) by mixing SNAP25-Cy3, synaptobrevin-TBD and a syntaxin SNARE helix peptide at equimolar ratios in 100 mM NaCl, 20 mM HEPES, 0.4% *n*-octylglucoside, pH 7.4. SNAP25-Cy3 was prepared by conjugating Cy3-NHS ester to the free cysteines of recombinant rat SNAP25. For injection into the tongue, mice were anesthetized with pentobarbital (30 mg/kg of body weight) and 1 μl of the same reagent was injected. Animals were placed in individual cages and 12 h later were intra-cardially perfused with 4% PFA, tissue dissected, post-fixed for 4 h and processed for histology.

Western blot

For liver and lung, tissues from the periphery of the organ devoid of large blood vessels or airway were harvested. Cortical tissue was from the frontal cortex. Approximately 30 mg of tissue isolated from all organs were disrupted in radioimmunoassay precipitation buffer (RIPA) buffer using a pestle in an Eppendorf tube, followed by further homogenization using 20 G needle and syringe. After sonication, the tissue lysates were incubated on ice for 30 min to extract the protein, clarified by centrifugation, and 20 μg of total protein homogenate quantified by BCA assay were loaded per lane onto a 10% acrylamide gel. The gel was run under constant 150 V for 1 h and dry transferred onto a nitrocellulose membrane. Membranes were blocked with 5% milk/TBST for 1 h, and incubated overnight with primary antibodies at 1:1000 mouse anti-S1R (#SC-166392, Santa Cruz Biotechnology, Dallas, TX, USA), 1:5000 mouse anti-β-actin (#A1978, Sigma–Aldrich, St. Louis, MO, USA), 1:10,000 mouse anti-GAPDH (#CB1001, EMD Millipore, Billerica, MA, USA) in 1% milk/TBST solution. Membranes were washed and incubated with 1:5000 of goat anti-mouse HRP (#31430, Thermo Fisher Scientific, Waltham, MA, USA) for 1 h at room temperature before imaging.