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THE SIGMA-1 RECEPTOR IS ENRICHED IN POSTSYNAPTIC SITES OF C-TERMINALS IN MOUSE MOTONEURONS. AN ANATOMICAL AND BEHAVIORAL STUDY

Timur A. Mavlyutov¹, Miles L. Epstein², Kristen A. Andersen¹, Lea Ziskind-Conhaim³, and Arnold E. Ruoho¹

Timur A. Mavlyutov: tamavlyutov@wisc.edu; Arnold E. Ruoho: aeruoho@wisc.edu

¹Department of Pharmacology, University of Wisconsin, School of Medicine and Public Health, 1300 University Ave, Madison, WI 53706, USA

²Department of Anatomy, University of Wisconsin, School of Medicine and Public Health, 1300 University Ave, Madison, WI 53706, USA

³Department of Physiology, University of Wisconsin, School of Medicine and Public Health, 1300 University Ave, Madison, WI 53706, USA

Abstract

The sigma-1 receptor regulates various ion channel activity and possesses protein chaperone function. Using an antibody against the full sequence of the sigma-1 receptor we detected immunostaining in wild type but not in knockout mice. The receptor was found primarily in motoneurons localized to the brainstem and spinal cord. At the subcellular level the receptor is restricted to large cholinergic postsynaptic densities on the soma of motoneurons and is colocalized with the Kv2.1 potassium channel and the muscarinic type 2 cholinergic receptor. Ultrastructural analysis of the neurons indicates that the immunostained receptor is located close but separate from the plasma membrane, possibly in subsurface cisternae formed from the endoplasmic reticulum (ER), which are a prominent feature of cholinergic postsynaptic densities. Behavioral testing on a rotarod revealed that Sigma-1 receptor knockout mice remained on the rotarod for significantly less time (a shorter latency period) compared to the wild type mice. Together these data indicate that the sigma-1 receptor may play a role in the regulation of motor behavior.

INTRODUCTION

The sigma-1 receptor is a 26 kD transmembrane protein found in the ER network of neurons and non-neuronal cells. In cultured cells and mammalian tissues it also has the ability to translocate from the endoplasmic reticulum (ER) to the plasma membrane or mitochondria-associated membranes (MAM) (Morin-Surun et al., 1999; Aydar et al., 2006; Hayashi and Su, 2007; Mavlyutov and Ruoho, 2007). During the last decade the view of sigma-1 receptor function has shifted to the idea that the receptor modulates cell membrane excitability by regulating the activity of K⁺, Ca²⁺, Na⁺, Cl⁻ ion channels (Lupardus et al., 2000; Zhang and Cuevas, 2005; Hayashi and Su, 2007; Renaudo et al., 2007; Johannessen et al., 2009). However, it remains to be firmly established whether ion channels are regulated directly or indirectly by

ligand-dependent conformations of the sigma-1 receptor. In this regard, among ion channels only the potassium Kv1.4 channel has been shown to form a direct complex with the sigma-1 receptor by coimmunoprecipitation experiments (Aydar et al., 2002).

The sigma-1 receptor binds to a variety of psychomimetic compounds (Su, 1982; Moebius et al., 1993; Nguyen et al., 2005) and neurosteroids (Su et al., 1988), all of which can bind to alternative targets. A variety of endogenous chemicals have been proposed to be natural ligands for the sigma-1 receptor including progesterone, pregnenolone (Su et al., 1988), dimethyltryptamine (Fontanilla et al., 2009), and sphingosine (Ramachandran et al., 2009). From a cell signaling prospective it is interesting that sphingosine, a non-psychotropic compound formed by sphingomyelin cleavage, also binds to the sigma-1 receptor (Ramachandran et al., 2009). Indeed it is possible that several endogenous compounds could be true ligands for the receptor depending on the cell type since the sigma-1 receptor is widely expressed in many tissues where the concentrations of the proposed endogenous molecules are likely to vary (Maurice and Su, 2009). The sigma-1 receptor has also been shown to possess chaperone activity (Hayashi and Su, 2007; Wu and Bowen, 2008).

The receptor has been localized to many areas in the rat brain via immunostaining (Alonso et al., 2000) and radioactive ligand binding (Gundlach et al., 1986; Walker et al., 1990; Bouchard and Quirion, 1997). However, the specificity of most anti sigma-1 antibodies has not been tested using knockout (KO) animals (Langa et al., 2003) as controls. Here we tested our antibody on wildtype (WT), and KO mice, using the KO mice as a negative control. We demonstrate that a high level of sigma-1 receptor is expressed in motoneurons of the brainstem and spinal cord. The data further indicate that within motoneurons the receptor is found in the ER and is also associated with cholinergic postsynaptic densities. Because of the relatively high concentration of the sigma-1 receptor in motoneuron cell bodies we tested motor functions in adult mice. We found differences in the motor behavior between the WT and sigma-1 KO mice.

RESULTS

Part I. Localization of sigma-1 receptors in the mouse brain

1. Distribution of sigma-1 receptors in the CNS—The first objective of this study was to identify regions of the CNS where sigma-1 receptors are expressed. We immunostained sections with anti-sigma-1 receptor polyclonal antibody (Fig. 1.A) (Ramachandran et al., 2007) and observed the receptor in the brainstem and spinal cord of two month old wild type mice but not in those from the knockout animals. The lack of immunostaining in knockout mice brain validates the specificity of our antibody. We found the highest level of sigma-1 receptor in the following nuclei: red nucleus, nucleus oculomotor, nucleus vestibularis lateralis, nucleus facialis, nucleus gigantocellularis, nucleus parvocellularis, nucleus hypoglossus and the lower motoneuron cell bodies in the ventral horn of all regions of the spinal cord (data not shown). Under experimental conditions reported here low labeling was seen in the olfactory bulbs, cerebral cortex, hippocampus or thalamus.

To further confirm our observations we examined sections of the spinal cord of HB9:eGFP transgenic mice (Wichterle et al., 2002) in which GFP is expressed in motoneurons and glutamatergic interneurons (Fig. 1B) (Hinckley et al., 2005; Wilson et al., 2005). We observed colocalization of GFP and the sigma-1 receptor in the ventral horn of the spinal cord. In addition, a lower level of labeling was found in some interneurons.

2. Subcellular distribution of sigma-1 receptors in motoneurons—The second objective was to determine the intracellular localization of sigma-1 receptors in the motoneurons. Evidence to date indicates that the sigma-1 receptor is localized to the ER in

both neural and non-neural cells, although a few studies show that the sigma-1 receptor can also be found close to or within the plasma membrane region (Morin-Surun et al., 1999; Aydar et al., 2006; Mavlyutov and Ruoho, 2007) and in the ER-mitochondrial contacts (Hayashi and Su, 2007). Although we observed the sigma-1 receptor in the ER of spinal motoneuron cell bodies, the most intense signal was detected as puncta in the regions adjacent to the plasma membrane (Fig.2.A). In order to determine whether the sigma-1 receptor was associated with synaptic terminals or randomly located within the plasma membrane, we co-stained ventral horn motoneurons with antibodies to the sigma-1 receptor and the presynaptic marker synaptotagmin (Fig.2.A). We found that the punctate distribution of the sigma-1 receptor was exclusively located adjacent to presynaptic terminals of motoneuron cell bodies and was absent from membrane regions lacking presynaptic input.

Motoneurons are densely innervated by excitatory and inhibitory synapses that include glutamate, acetylcholine, serotonin, catecholamines, GABA and glycine. To determine the identity of the presynaptic neurotransmitters impinging on the sigma-1 receptor positive postsynaptic densities, we applied a variety of antibodies that are specific markers for the above neurotransmitter synapses. We found that the sigma-1 receptor is juxtaposed to presynaptic terminals labeled with antibody specific for the vesicular acetylcholine transporter (Fig.2.B) and postsynaptically colocalized with the metabotropic acetylcholine receptor m2 (Fig.3.A). Thus we conclude that the sigma-1 receptors are located in postsynaptic densities on ventral horn motoneuron cell bodies exclusively under cholinergic synapses known as C-terminals (Nagy et al., 1993). Previous work has shown the sigma-1 receptor can regulate the activity of potassium (Lupardus et al., 2000; Aydar et al., 2002), sodium (Johannessen et al., 2009), calcium (Hayashi and Su, 2007) and chloride channels (Renaudo et al., 2007). Because the Kv1.4 potassium channels are known to be extensively modulated by the sigma-1 receptor (Aydar et al., 2002), we investigated whether these channels are also present in motoneurons and if they are colocalized with the sigma-1 receptor. Immunolabeling failed to reveal the presence of Kv1.4 (data not shown). However, the delayed inward rectifier Kv2.1 channel which is known to be associated with the C-terminals in motoneurons (Muennich and Fyffe, 2004) colocalized with the sigma-1 receptor in the C-terminals (Fig.3.B). The absence of the sigma-1 receptor in the ventral horn motoneuron cell bodies of KO animals, however, did not alter significantly the distribution of Kv2.1 (Supplemental Fig.1).

Using horseradish peroxidase to localize the receptor at the ultrastructural level (Fig.4.top panel), we found an electron-dense precipitate in postsynaptic densities adjacent to large cholinergic presynaptic terminals. At high magnification the receptors were slightly separated from the plasma membrane (Fig.4.bottom panel) which is consistent with the sigma-1 receptor localization in subsurface ER cisternae and not in the plasma membrane (Nagy et al., 1993). Cholinergic C-terminals on the cell bodies of motoneurons are the largest compared to other terminals (at least 1–2 μm in length) and have been previously reported to contain well-developed subsurface ER cisternae, which are characteristic of these cholinergic postsynaptic densities (Yamamoto et al., 1991; Nagy et al., 1993). In order to support the association of the sigma-1 receptor and the subsurface ER cisternae, we found evidence that the general ER marker calsequestrin is colocalized with the sigma-1 receptor in postsynaptic sites of C-terminals (Supplemental Fig.2). For the sake of clarity we provide a diagram with the sigma-1 receptor localized to subsurface cisternae in cholinergic postsynaptic density of motoneuron (Fig.5).

Part II. Behavioral assays

Since we located the sigma-1 receptor in motoneurons, we assessed the effects of the absence of the receptor on motor behavior. For this purpose wildtype (WT) $n=10$ and knockout (KO) $n=16$ animals were tested. The weight of each mouse was recorded weekly starting from

postnatal week 8. Throughout the duration of the study no statistically significant differences in weight were noted between the two genotypes ($p=0.795$) (Supplemental Fig.3).

1. Rotorod assays—The rotorod test is considered to be one of the most reliable tests to estimate motor coordination skills (Brooks and Dunnett, 2009). During the assay, a rod-like beam is rotated at various speeds and the time (latency) that the mouse maintains balance on the beam before falling off is recorded. This experiment was repeated on a weekly basis to study the motor coordination development of the two mouse genotypes over time. It was observed that at the three experimental settings used the KO mice showed the shortest latency comparing to WT mice (Fig.6A) : 15 rpm ($p=.0051$), 20 rpm ($p=0.0097$), accelerating speed 4–45 rpm ($p=0.0018$). Over the course of the study we observed that all the animals showed an increase in latency but the differences between the genotypes remained.

2. Swimming—The swimming test is considered a sensitive assay for the coordinated movement of limbs (Brooks and Dunnett, 2009). We measured the time for the mice to swim from a line in a water tank to a dry safety platform. KO mice consistently swam faster than WT mice (Fig.6B) ($p=0.0001$). This result was unexpected and so we studied the patterns of movement during each trial. While all mice used both rear paws as a source of locomotion during swimming, the WT and KO mice used their front paws in a different manner. The KO mice appear to use either one or both front paws sparingly and for steering purposes only, whereas the WT mice used both front paws for locomotion purposes. In addition, the KO mice appeared to use their tail for propulsion while the WT mice maintained their tails above the water surface.

3. Gait study—The footprint test analysis is considered to be a sensitive assay for motor coordination (Brooks and Dunnett, 2009). Our results showed no difference in the stride length between WT and KO ($p=0.2478$) (Supplemental Fig.4).

DISCUSSION

Using a specific polyclonal antibody to the sigma-1 receptor (Ramachandran et al., 2007), we observed the sigma -1 receptor to be localized exclusively to motoneurons in the mouse brainstem and spinal cord but to be absent from sigma-1 knockout mice. The receptor positive neurons were likely to be motor neurons by the colocalization of the sigma-1 receptor and GFP expression directed by the motoneuron specific HB9 promoter (Wichterle et al., 2002). Our localization results are quantitatively in contrast to those of Alonso et al. (Alonso et al., 2000), who observed receptor labeling in cells lining the ventricles and in neurons in the olfactory bulb, hippocampus, amygdala, cortex, and the dorsal horn of the rat spinal cord. We found low immunoreactive signal in those areas reported by Alonso et al (Alonso et al., 2000). Although Alonso reported faint labeling in the spinal cord ventral horn, our data indicated heavy labeling in motoneurons of the brainstem and spinal cord. (Fig.1.A).

A number of possibilities could explain the difference: 1) rats may have a different distribution of sigma-1 receptor in the aforementioned regions of brain compared to mice. However, C-terminals are anatomically similar in rats and mice (Hellstrom et al., 1999; Muennich and Fyffe, 2004; Wilson et al., 2004). 2) The specificity of antibody also could be significant. In the Alonso's et al. study their antibody was generated against a 20 amino acid peptide (fragment 143–162) located in the putative ER intraluminal region of the sigma-1 receptor (Alonso et al., 2000). In contrast, our antibody was raised against the full sequence of the sigma-1 receptor and may recognize its native conformation (Ramachandran et al., 2007), thus providing higher specificity for recognition of the sigma-1 receptor in situ. The absence of signal in the KO animals further indicated that our antibody is specific for the sigma-1 receptor. However, we

still cannot disregard the possibility that the affinity of anti-sigma-1 receptor antibodies might not be high enough to detect all sigma-1 receptor-enriched regions.

Using a series of neurotransmitter markers to explore the properties of the receptor, we demonstrated via confocal microscopy the presence of the sigma-1 receptor in cholinergic postsynaptic densities by colocalization with the m2 receptor and its juxtaposition to the pre-synaptic terminal by presence of the vesicular associated acetylcholine transporter (Fig.2). Although we were unable to determine the precise location of the sigma-1 receptor because of the limitations of the preembedding staining procedure and diffusion of the precipitate, our images at the EM level are consistent with its location in the subsurface ER cisternae in motoneuron cell bodies and not in the plasma membrane (Fig.4 and Fig.5). C-terminals in motor neurons were recently shown to receive input from a subset of lamina X interneurons, thought to be involved in central pattern generation (Miles et al., 2007; Zagoraïou et al., 2009). It is not clear at present if the sigma-1 receptor located in subsurface cisternae of postsynaptic sites of C-terminals influences motoneuron activity. We can only speculate that ion channel conductivity may be modulated by either direct or indirect actions of the sigma-1 receptor. Direct action could result from interaction of the sigma receptor with the Kv2.1 or other ion channels. Indirect action could occur by increases in intracellular calcium resulting from its release from subsurface cisternae. Ultimately changes in ion channel conductivity changes the pattern of motoneuron firing, which may result in behavioral changes. In C-terminal postsynaptic densities subsurface cisternae are very well developed and are connected to the entire network of the ER (Nagy et al., 1993; Li et al., 1995). We speculate that the approximately 10 nm distance between the subsurface cisternae and the plasma membrane may allow direct interaction between proteins on the plasma membrane and those on the subsurface cisternae (ie. *in trans*). Such interactions have been observed between STIM and CRAC channels (Clapham, 2009) and between voltage-sensitive dihydropyridine receptors on muscle T-tubules and ryanodine receptors on the lateral sac of the sarcoplasmic reticulum (see also Fig.5). Previous work has shown the sigma receptor interacts with and can regulate the activity of the Kv1.4 potassium channels (Aydar et al., 2002; Mavlyutov and Ruoho, 2007). In this work we also demonstrated the colocalization of both the Kv2.1 potassium channels and the muscarinic m2AChR with the sigma-1 receptor. Although they appear colocalized, our resolution is limited so that they may lie in different membranes. It is possible that the sigma-1 receptor regulates either or both the plasma membrane bound Kv2.1 potassium channel and/or M2 muscarinic receptor via protein-protein interactions from the ER cisternae. Behavioral differences between sigma-1 KO mice and WT mice were only observed after the injection of sigma-1 ligands (Langa et al., 2003; Fontanilla et al., 2009). In our study we showed that even without ligand application, the latency on the rotarod was significantly shorter for KO than for WT mice. These data suggest that some component(s) in neuronal pathways controlling motor coordination of the mouse are impaired in the absence of the sigma-1 receptor. The rotarod measures balance and motor coordination. Unexpectedly the swimming performance which also evaluates motor coordination (Brooks and Dunnett, 2009) of KO mice was significantly better than WT counterparts. We observed differences in the pattern of swimming locomotion. The knockouts use their tail but not their front paws in swimming while the wildtypes use both front and back limbs but not tails (see supplemental movie). Although it is not clear if these differences result in faster swimming performance, we speculate that the different swimming behavior is another manifestation of the lack of sigma receptors. Considered together it is reasonable to conjecture that the lack of the sigma-1 receptor in the cell bodies of motoneurons in cholinergic postsynaptic densities and/or in the red nucleus, vestibular nucleus, nucleus gigantocellularis or even interneurons may underlie these differences in motor behavior.

MATERIALS AND METHODS

SOURCE OF ANIMALS

Opr^s1 mutant (+/−) Opr^sGt(IRESBetageo)^{33Lex} litters on a C57BL/6J × 129s/SvEv mixed background were purchased from the Mutant Mouse Regional Resource Center, UC Davis, CA. All mice were maintained on a normal 12-hour light/dark cycle and handled in accordance with animal care and use guidelines of the University of Wisconsin, Madison.

ROTOROD

Two month-old wildtype and sigma-1 knockout mice were tested on a rotorod apparatus (IITC Life Science) each week for 8 weeks. Each mouse was placed in the forward position on a rotating beam with a diameter no smaller than 1.25 inches, rotating with either a constant speed of 15 rpm, or 20 rpm or acceleration from 4 to 45 rpm over a period of 5 minutes. The beam was suspended no higher than 12 inches above a cushioned box. Tests were performed in duplicate, cycling through all the mice in a test group before retesting. This experimental paradigm provided a rest time of 40–45 minutes for the mice between trials, during which the animals had free access to food and water. Mice were tested each week at the same time of the day during 8–14 weeks of postnatal life to follow changes in agility and motor coordination.

SWIMMING

Mice were trained as described below to swim in a water tank from one end to another. The tank was custom-made from Plexiglas with dimensions: 100 cm long, 35 cm tall, 6 cm wide. Vertical black lines were drawn 20 cm from each end of the tank to mark a 60 cm measurement zone. A safety zone platform, constructed of a 20 cm dark platform, was added to one end of the tank at a height of 20 cm above the base of the tank. The other section of the tank excluded by the black line was considered the orientation zone, in which the mouse adapted to the situation prior to being measured. Mice were placed in the orientation zone and allowed one adaptive trial, which ended when the safety zone was reached. Three measured experimental trials were given to each mouse, with a rest period of 30 seconds between each trial. Measurements were initiated when the nose of the mouse crossed the first black line and completed when the front paws reached the safety zone. The patterns of swimming movements were also recorded.

GAIT STUDY

To study any possible differences in stride, the hind feet of the mice were painted black using acrylic non-toxic paint. Mice were then introduced into a custom-made tunnel made from transparent Plexiglas with dimensions: 30 cm long, 4 cm tall, 3 cm wide. A dark box was fixed at the end of tunnel as an attraction for the mice to move forward. A sheet of paper was placed on the walking base each time that a new mouse was tested. Stride length was measured in centimeters as the average distance between consecutive left and right footprints. The assessor of behavioral study was aware of the genotype of mice in all behavioral assays reported in this study.

IMMUNOCYTOCHEMISTRY

Mice were anesthetized with Nembutal and perfused through the left ventricle with phosphate buffered saline (PBS) containing heparin followed by 4% paraformaldehyde +/− 0.1% glutaraldehyde for 30 min. The brain and spinal cord were then dissected and postfixed with the same fixative overnight. The tissue was rinsed for 10 h in PBS, and cryoprotected in 30% sucrose in PBS for 48 h, all at 4°C. The tissue was cut on a sliding freezing microtome at 60 μm, collected in PBS, permeabilized with 1% Triton X-100 for 30 min, blocked with 10% normal goat serum and stained with various dilutions of rabbit anti-sigma serum (1/300 – 1/700) and

with goat-anti-rabbit conjugated horseradish peroxidase (Sigma-Aldrich) (1/500–1/1000) antibodies, and visualized by subsequent incubation in 1.4 mM 3, 3-diaminobenzidine and 5.3 mM imidazole in 0.2 M Tris buffer pH 7.6.

For fluorescent microscopy, sections 7–8 µm thick were cut on a cryostat. Some sections were also cut on a vibratome (EMS sciences) at 60 micron thickness and the following procedure was performed on free-floating slices. Sections were blocked in 10% normal goat serum and primary antibodies were applied for 48 h. A mixture of anti-sigma-1 antibody at a concentration 0.25 µg/ml and a variety of monoclonal antibodies at concentrations 1–3 µg/ml were applied to sections. Sections were rinsed and secondary antibodies of Alexa-488 conjugated goat-anti-rabbit and Alexa 568 conjugated goat-anti-mouse at concentration 2 µg/ml were applied overnight. Sections were rinsed, counterstained with DAPI and embedded into Prolong Gold mounting media (Invitrogen) and coverslipped. Images were collected on a Zeiss Axiovert 200 M epifluorescent microscope with a 100× oil objective with Axiovision 4.3 software. Some images were taken with Nikon A1R laser confocal microscope (Nikon, Tokyo, Japan) supplied with green 488 nm Argon laser, red 561 nm DPSS laser through a Apo60× VC oil-immersion objective with NIS elements software. Acquired Z-stacks were analyzed with Image J software for single plane selection. Single planes were saved as TIF files and further minor adjustments for brightness and contrast were applied in Adobe Photoshop. Final figures were made in Adobe Illustrator.

The following antibodies were used (also see supplemental table): rabbit polyclonal anti sigma-1 (Ramachandran et al., 2007) with a dilution range (1:400–1:700), and monoclonal antibodies from Antibodies Incorporated (UC Davis/NIH NeuroMab Facility, CA) anti-VACHT (3 µg/ml, #75-020), anti-Kv2.1 (3 µg/ml, #75-014). Monoclonal antibody against the m2AChR was obtained from “Chemicon” (#MAB367). Monoclonal antibody against synaptotagmin was a gift from Dr. E. Chapman, Department of Physiology, University of Wisconsin, Madison. Fluorescently conjugated secondary antibodies were from Invitrogen: goat-anti-rabbit Alexa488 conjugated Fab fragment (#A11070), goat-anti-mouse Alexa568 Fab fragment (#A11019), goat-anti-rat IgG Alexa 568 (#11077) were all applied at 2 µg/ml.

IMMUNOELECTRON MICROSCOPY

For preembedding immunoelectron microscopy vibrotome slices 60 µm thick of spinal cord from perfused mice were treated with 0.1% NaBH₄ for 10 min, washed in PBS and permeabilized with 0.3% saponin. This detergent was also used during incubation with primary and secondary antibodies and washing steps. Slices were blocked in 10% goat serum for 4 hours, washed and rabbit anti-sigma-1 antibody were applied (2 µg/ml) for 48 hours. Samples were then extensively washed and secondary goat-anti-rabbit HRP conjugated antibody (diluted 1/200) were incubated overnight. Samples were washed and developed in 3, 3-diaminobenzidine and imidazole for 3 min. Samples were washed, postfixed in 2.5% glutaraldehyde, washed and silver enhanced for 15 min, washed, poststained with 0.5% OsO₄, dehydrated in series of alcohols, embedded into EPON and cut at 100nm. Samples were briefly stained with uranyl acetate and lead citrate. Images were collected with Philips CM120 electron microscope.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Distribution of sigma-1 receptor in the mouse CNS. **A.** In the mouse brain the greatest staining for the sigma-1 receptor appeared in the medulla and the spinal cord. The heaviest labeling is detected in the motor nuclei. No antibody labeling appeared in brain and spinal cord sections from the sigma-1 KO mice. **B.** Colocalization of sigma-1 receptor and the motoneuron marker Hb9 derived GFP. **a.** Sigma-1 is highly expressed in the ventral horn in motoneurons of Hb9 transgenic mice. **b.** Motoneurons expressing Hb9 derived GFP. **c.** Merged image shows sigma-1 is confined to motoneurons. **d.** Merged image overlapped with interference contrast optic image.

Figure 2.

In the motoneurons the sigma-1 receptor was enriched in cholinergic postsynaptic densities.

A. Presynaptic marker synaptotagmin (syt) and sigma-1 receptor are juxtaposed but do not overlap, indicating the sigma-1 is not in presynaptic boutons. **B.** Vesicular associated acetylcholine transporter (VACHT) positive presynaptic boutons and sigma-1 enriched postsynaptic densities are separated but adjacent, showing that in the plasma membrane the sigma-1 is found in cholinergic postsynaptic sites on cell bodies.

Figure 3.

Sigma-1 receptor colocalized with receptors known to be located on postsynaptic side in cholinergic C-terminals. **A.** The metabotropic muscarinic acetylcholine receptor (m2) and the sigma-1 receptor colocalized, indicating the postsynaptic location of sigma-1. **B.** All large clusters Kv2.1 potassium channels and sigma-1 receptors colocalized.

Figure 4.

Ultrastructural visualization of sigma-1 receptor. **Top panel.** Electron-dense precipitate from HRP immunostaining appears in the postsynaptic sites adjacent to large presynaptic boutons. Asterisk indicates the presynaptic side; P indicates the postsynaptic side, body of the motoneuron. Electron-dense precipitate appears on the postsynaptic side close to the plasma membrane. **Bottom panel.** Higher magnification of the synaptic contact on the body of motoneuron from the top panel. The dense signal appears separated from the plasma membrane and is likely to be in subsurface ER cisternae which are known to be well-developed at postsynaptic sites of C-terminals. The detergent treatment has partially compromised the structural integrity of motoneuron cell bodies. Black arrowheads point to the presynaptic

membrane; open arrowheads point to the postsynaptic membrane; arrows point to the electron-dense precipitate formed by the DAB deposit. Note that the sigma-1 signal is located close but apart from the plasma membrane.

Figure 5.
A diagram illustrating that in cholinergic postsynaptic densities of motoneuron cell bodies the sigma-1 receptor is located on the subsurface cisternae in close apposition to the plasma membrane.

Figure 6.

Behavioral changes in sigma-1 KO mice. **A.** Rotorod behavior. Sigma-1 receptor KO mice showed impaired performance on the rotorod. At all three settings of the rotorod (constant speed 15 rpm and 20 rpm, or accelerating speed 4–45 rpm) KO mice showed less latency than the WT mice. Experiments were performed weekly at postnatal weeks 8–14. WT (n=10), KO (n=16). **B.** Swimming performance. Sigma-1 receptor KO mice swam to the safety zone faster than WT mice. WT (n=10), KO (n=16). Error bars: standard deviation.