The Sigma Receptor: Evolution of the Concept in Neuropsychopharmacology

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Abstract: Although originally proposed as a subtype of opioid receptors, the sigma receptor is now confirmed to be a non-opioid receptor that binds diverse classes of psychotropic drugs. Sigma receptors are subdivided into two subtypes, sigma-1 and sigma-2. The sigma-1 receptor is a 25-kDa protein possessing one putative transmembrane domain and an endoplasmic reticulum retention signal. Sigma-1 receptors are highly expressed in deeper laminae of the cortex, olfactory bulb, nuclei of mesencephalon, hypothalamus, and Purkinje cells in the brain. Sigma-1 receptors are predominantly localized at the endoplasmic reticulum of both neurons and oligodendrocytes. From behavioral studies, sigma-1 receptors were shown to be involved in higher-ordered brain functions including memory and drug dependence. The actions mediated by sigma-1 receptors at the cellular level can be considered either as acute or chronic. The acute actions include the modulation of ion channels (i.e., K⁺ channel, NMDA receptors, IP₃ receptors) and the sigma-1 receptor translocation. Chronic actions of sigma-1 receptors are basically considered to be the result of an up- or down regulation of the sigma-1 receptor itself. For example, the upregulation of sigma-1 receptors *per se*, even without exogenous ligands, promotes cellular differentiation and reconstitution of lipid microdomains (lipid rafts) in cultured cells. These findings together suggest that sigma-1 receptors might possess a constitutive biological activity, and that sigma-1 receptor ligands might merely work as modulators of the innate activity of this protein. Recent *in vitro* and *in vivo* studies strongly point to the possibility that sigma-1 receptors participate in membrane remodeling and cellular differentiation in the nervous system.

Key Words. Sigma receptor, sigma-1 receptor, potassium channel, IP₃ receptor, lipid rafts, differentiation, oligodendrocyte, drug dependence.

INTRODUCTION

Based on behavioral observations, Martin *et al.* proposed a subtype of opioid receptors as the sigma/opioid receptor [40]. In contrast to causing analgesia such as that caused by morphine, benzomorphans including (+)SKF10047 (N-allyl-normetazocine) and pentazocine can both cause psychotomimesis in the dog [40]. Martin suggested therefore that *sigma opioid receptors* contribute to delusion and psychosis induced by opioids [40]. Su, by using the tritiated prototypic *sigma opioid receptor* ligand SKF-10047 in a binding assay, identified a protein that has a nanomolar affinity for SKF-10047 [79]. Unexpectedly, the SKF-10047-binding protein has no affinity for the opioid antagonist naloxone. This finding raised a possibility that the protein identified by Su may not be the *sigma opioid receptor* proposed by Martin. Furthermore, the SKF-10047-binding site as shown by Su has higher affinity for dextrorotatory benzomorphans like (+)SKF-10047 and (+)pentazocine than for levorotatory isomers [79]. This stereospecificity is opposite to that seen with all opioid receptor subtypes in either binding assays or behavioral tests, further supporting that the SKF-10047-binding protein is not an opioid receptor [79]. The protein identified by Su was later termed "sigma receptor", instead of *sigma opioid receptors*, to distinguish the protein from opioid receptors.

After the identification of sigma receptors, confusion between the PCP site of the NMDA receptor and sigma receptors occurred because sigma ligand (+)SKF1007 binds the PCP site as well, and, conversely, phencyclidine has an appreciable affinity for sigma receptors [91]. However, later binding assays using selective ligands for respective binding sites (e.g., (+)pentazocine for sigma receptors and TCP for PCP-sites) successfully differentiated sigma receptors from the PCP site [14, 37, 38, 87]. The autoradiographic studies also demonstrated the differential distributions of the sigma binding site and the PCP site in the brain [14, 37]. These extensive ligand binding studies finally led to a conclusion that sigma receptors are non-opioid, non-PCP, brain receptors [66]. Thereafter, (+)pentazocine replaced (+)SKF10047 as a prototypic sigma receptor ligand.

Bowen and colleagues further examined the characteristics of (+)pentazocine binding to sigma receptors [25]. They discovered that sigma receptors could be subdivided into two subtypes: sigma-1 receptors and sigma-2 receptors [25]. Regarding the sigma-1 receptor thus found, the similar ligand binding profile [25], specifically the higher affinity of dextrorotatory benzomorphans versus the levorotatory isomers at sigma-1 receptors, indicate that the sigma-1 receptor subtype is the same as originally described by Su [79]. On the other hand, sigma-2 receptors as identified by Bowen *et al.* [25] are characterized by the levorotatory benzomorphans being equal or more potent than their counterpart dextrorotatory isomers [9]. Recent studies demonstrate that the two respective subtypes mediate different cellular and physiological functions, although a few functions are mediated by both. Since the successful cloning

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of the sigma-1 receptor in 1996 [15], the function of sigma-1 receptors has been more extensively examined in different research areas (e.g., cell and molecular biology, cancer, immunology, and behavioral neuroscience). In this review, the focus is on the sigma-1 receptor and recent developments in defining the functions of sigma-1 receptors at cellular, physiological and behavioral levels. These recent findings represent an emerging concept and relevant perspectives on the role of sigma-1 receptors in the biological system.

1. SIGMA LIGANDS (TABLE 1)

Of peculiar note regarding the ligand binding profile of the sigma-1 receptor is its multi drug-binding spectrum. Although the sigma-1 binding site was originally identified by dextrorotatory benzomorphans like (+)SKF-10047 and (+)pentazocine, later binding studies demonstrated that the sigma-1 receptor binds diverse classes of pharmacological compounds [14, 25, 37, 38, 66, 79, 87, 91]. In addition to benzomorphans, the sigma-1 receptor binds haloperidol, imipramine, fluvoxamine, pimozone, chlorpromazine and dextromethorphan in *in vitro* binding assays [22, 79]. Specifically, a majority of antipsychotics possess high to moderate binding affinities for sigma-1 receptors [22, 79]. However, some atypical antipsychotics such as sulpiride and clozapine do not bind to sigma-1 receptors [82]. These findings suggest that the affinity of antipsychotics for the sigma-1 receptor might, at least in part be related to their side effects (e.g., extrapyramidal symptoms). The micro-injection of sigma ligands in the red nucleus was shown indeed to cause acute dystonia in animals [94]. Neurosteroids like sex hormones progesterone, testosterone, and pregnenolone sulfate have moderate affinity at sigma receptors, and are proposed as candidates for endogenous sigma-1 ligands [80]. Recent behavioral studies demonstrated that some neurosteroids could induce pharmacological actions *via* the sigma-1 receptor *in vivo* [45, 48]. Nevertheless, neurosteroids as endogenous sigma-1 ligands have not been confirmed and remain elusive. Although sigma-1 receptors are shown to bind diverse classes of compounds in *in vitro* binding assays [79], it is still unclear whether all ligands can produce pharmacological actions *via* sigma-1 receptors when they are administered to animals. Only a few sigma-1 receptor ligands were tested in *in vivo* systems in this regard [63]. Because the action site of sigma-1 ligands is supposed to be mainly located at the intracellular organelle endoplasmic reticulum (see "Cellular distribution of sigma-1 receptors"), all ligands may not reach sufficient concentrations to occupy sigma-1 receptors. This issue is particularly important for the drug development and when using so-called sigma-1 receptor ligands to treat animals or humans.

2. GENE AND STRUCTURE OF SIGMA-1 RECEPTORS (FIG. 1))

Since the first cloning of guinea-pig sigma-1 receptors [15], sigma-1 receptor genes have been cloned from several mammalian cDNA libraries [33, 70, 72]. The sigma-1 receptor was first purified as a single 30-kDa protein from the guinea pig liver employing benzomorphan (+)[³H]pentazocine and arylazide (-)[³H]azidopamil as specific probes [15]. Radiation inactivation of the pentazocine-

Table 1. Sigma-1 Receptor Ligands

Compounds	Subtype selectivity	Function
Benzomorphan		
(+)SKF-10047	sigma-1	agonist
(+)Pentazocine	sigma-1	agonist
Dextromethorphan	sigma-1	agonist
(+)-3-PPP	sigma-1	agonist
Antipsychotic		
Haloperidol	sigma-1/2	antagonist
Spiperone	sigma-1/2	?
Pimozide	sigma-1?	?
Fluphenazine	?	?
Chlorpromazine	?	?
Nemonapride	sigma-1/2?	?
Antidepressant		
Imipramine	sigma-1	agonist
Fluoxetine	sigma-1	agonist
Fluvoxamine	sigma-1	agonist
Neurosteroid		
Progesterone	sigma-1	antagonist
Testosterone	sigma-1	?
DHEA-sulfate	sigma-1	agonist
Other synthetic compounds*		
DTG	sigma-1/2	agonist?
BD-1008	sigma-1/2	antagonist
PRE-084	sigma-1	agonist
NE-100	sigma-1	antagonist
JO-1784	sigma-1	agonist
SA-4503	sigma-1	agonist
SR-31747A	?	?
SR-31742A	sigma-1	antagonist
MS-377	sigma-1	antagonist
BMV-14802	sigma-1	antagonist
Panamesin	sigma-1?	antagonist?
E-5842	sigma-1	antagonist

*Major ligands that possess affinities for sigma-1 receptors higher than 500 nM are only listed.

labeled sigma-1-binding site yielded a molecular mass of 24 +/-2 kDa that is consistent with the molecular weight predicted from the sigma-1 receptor cDNA open reading

Fig. (1). Protein structure of the sigma-1 receptor.

frame (25.3-kDa; 223 amino acids) [15]. The cloned sigma-1 receptor reveals no homology to any known mammalian protein [15, 33, 70, 72]. The amino acid sequence of sigma-1 receptors exhibits more than 90% identity in different mammalian species including guinea pig, human, rat and mouse [33, 70, 72]. The protein expressed in yeast with the cloned cDNA showed the same pharmacological characteristics as those seen with the brain and the liver sigma-1-binding site [15]. The cloned cDNA, when functionally expressed in mammalian cells, enhances the binding of sigma-1 receptor ligands. Particularly, transfection of MCF-7 cells, which do not express type-1 sigma receptor mRNA or activity, with the cloned rat brain cDNA leads to the appearance of haloperidol-sensitive binding of (+)-pentazocine [70]. These studies confirmed that the cloned cDNA encodes the pharmacologically identified sigma-1 binding site.

Sigma-1 receptors contain three hydrophobic domains, namely, at the N- and the C-termini, and at the center of the protein (Fig. (1)). The original cloning study predicted the N-terminal hydrophobic domain as a potential transmembrane domain [15], but later reports suggested the hydrophobic domain in the middle of the protein could be the transmembrane domain [33, 70, 72]. Recently, a topology model was proposed where the sigma-1 receptor may have two transmembrane domains near the N-terminus and the center of the protein, whereas both the N- and the C-termini are cytosolic [4]. However, more extensive studies should be performed to a conclusive topographic structure of the sigma-1 receptor.

The deduced amino acid sequence of the sigma-1 receptor reveals the presence of a double-arginine endoplasmic reticulum retention signal at the N-terminus of the sigma-1 receptor [15, 33, 70, 72]. This fact indicates that the sigma-1 receptor protein may reside on the endoplasmic reticulum membranes. Although the amino acid sequence of the sigma-1 receptor is structurally unrelated to known mammalian proteins, it shares a homology with a fungal protein C8-C7 sterol isomerase that is involved in sterol synthesis [15]. This

finding raises one possibility that sigma-1 receptors might be related to enzymes involved in sterol biosynthesis [15]. The possibility indeed is in agreement with the fact that the sigma-1 receptor's affinities for steroids, such as progesterone are in a nanomolar range [80]. However, when expressed in yeast lacking the C8-C7 isomerase gene, the sigma-1 receptor cannot restore the enzymatic activity of sterol isomerase [15, 35, 51]. Further, overexpression of sigma-1 receptors in mammalian cells does not even significantly affect cholesterol biosynthesis [23, 86]. The knock down of sigma-1 receptors by siRNAs did not affect total cholesterol levels in oligodendrocytes [21]. Recently, mammalian C8-C7 isomerase was cloned and shown to have a totally different structure to that of the sigma-1 receptor [6]. Thus, the exact constitutive biological activity of the sigma-1 receptor, if any, is unknown at present.

The entire sequence of the sigma-1 receptor gene, the exon-intron organization and the promoter region sequence for transcription factor binding sites, have been extensively examined [33, 70, 72]. The gene (approximately 7 kb) is TATA-less but contains CCAATC and GC boxes immediately upstream of the transcription starting site in the mouse sigma-1 receptor gene [72]. The gene consists of 4 exons and 3 introns [70]. Recently, a form of splicing variants lacking exon 3 is reported [70]. The 5'-flanking region contains putative binding sites for AP-1, AP-2, GATA-1 and steroid receptors [72]. There are also consensus sequences for the liver-specific transcription factor nuclear factor-1/L, for a variety of cytokine responsive factors, and for the xenobiotic responsive factor called the arylhydrocarbon receptor [72]. Southern blot analysis of the genomic DNA has shown that the gene is located on human chromosome 9, band p13, a region known to be associated with different psychiatric disorders [65].

Since it is known that a splice variant of the sigma-1 receptor which lacks exon 3 does not bind sigma ligands [12, 70], the ligand-binding domain was speculated to be present in or around the region encoded by exon 3. Ganapathy and colleagues explored the ligand binding domain on the sigma-

1 receptor by provoking point mutations in each of the anionic amino acids in this region and assessed the influence of each mutation on the ligand binding [71]. This study has identified two anionic amino acids, Asp126 and Glu172, that are obligatory for ligand binding [71]. Yamamoto *et al.* also examined amino acid sequence responsible to sigma ligand binding, especially focusing on the hydrophobic domain in the middle of the protein [97]. They expressed sigma-1 receptors in *Xenopus* oocytes and assessed binding abilities of the sigma-1 ligands [³H](+)-pentazocine and [³H]NE-100 (N,N-dipropyl-2-[4-methoxy-3-(2-phenyl-ethoxy)-phenyl]-ethyl-amine-monohydrochloride). Amino acid substitutions (Ser99Ala, Tyr103Phe and di-Leu105,106di-Ala) in the transmembrane domain did not alter expression levels of the sigma-1 receptor when assessed by Western blottings, however, ligand binding was significantly suppressed by the substitutions [97]. These findings provide evidence that the hydrophobic domain in the center of the sigma-1 receptor also plays a role in the ligand binding property of this receptor. Findings from these two independent studies suggested that multiple domains on the sigma-1 receptor are required for sigma ligand bindings (Fig. (1)). Perhaps, a 3-D structural configuration, if available, and/or a possibility of protein oligomerization may support existence of multiple domains that are important for ligand-binding sites and for the trans-drug class binding property of this receptor.

3. DISTRIBUTION OF THE SIGMA-1 RECEPTOR

3.A. Body and Brain Distribution of Sigma-1 Receptors

Northern blot analysis indicated that the sigma-1 receptor-specific transcript is expressed abundantly in the liver and moderately in the intestine, kidney, white pulp of the spleen, adrenal gland, brain, placenta and the lung [70, 71, 98]. Several cancer cell lines express the sigma-1 receptor mRNA to a variable extent [33]. In *in situ* hybridization, specific sigma-1 receptor hybridization signals were found to be widely, but discretely distributed in mouse and guinea pig brain tissues [34, 71, 98]. The highest levels of transcripts were observed in cranial nerve nuclei [34]. High hybridization signals were also observed in mesencephalon such as the red nucleus, periaqueductal gray matter and substantia nigra, as well as in some diencephalic structures including paraventricular and ventromedial hypothalamic nuclei [34]. Superficial (I-II) and deeper (IV-VI) cortical laminae were moderately labeled in the mouse brain [34]. Moderate levels were detected in the pyramidal cell layer and the dentate gyrus of hippocampus [34]. Overall, the sites of expression of specific sigma-1 receptor mRNA signals were in accordance with the anatomical distribution of the sigma-1 receptor protein first established by quantitative receptor autoradiography [34]. In the postmortem human brain, moderate levels of the sigma-1 receptor mRNA, distributed in a laminar fashion, were detected in the temporal cortex with the deeper laminae (IV-VI) being particularly enriched [34]. In the hippocampus, the strongest hybridization signals were observed in the dentate gyrus, while all other subfields of the human hippocampus expressed lower levels of the sigma-1 receptor mRNA [34].

Overall expression patterns of sigma-1 receptor proteins are fairly consistent with results from the mRNA and radioligand binding assays with a few discrepancies (Fig. (2)). Immunohistochemistry showed quite high levels of sigma-1 receptors in olfactory bulb and hypothalamus, but much lesser are seen in cortex and hippocampus [1, 60, 64]. In the cerebellum, sigma-1 receptors are specifically expressed in Purkinje cells (Fig. (2)). Faint immunostaining was associated with other areas in the cerebellum and with neurons located in the caudate-putamen [1, 60, 64]. Sigma-1 receptors are also highly expressed in the dorsal horn of spinal cord [1] and in the ependymocytes on the surface of ventricles (Fig. (2)) [1, 21]. In cortical structures, the sigma-1 receptor immunostaining was associated with the perikarya and dendrites of pyramidal neurons located in layers II–III and V of the parietal cortex, with overall results consistent with those of the mRNA [1, 60, 64] (Fig. (2)). Recently, sigma-1 receptors have been shown to regulate certain functions of retina [7]. Immunohistochemical studies detected the sigma-1 receptor protein in the retinal ganglion, photoreceptor, RPE cells, and in the surrounding of the soma of cells in the inner nuclear layer [65].

3.B. Cellular Distribution of Sigma-1 Receptors

Early studies combining the subcellular fractionation with the radioligand binding assay indicated that sigma-1 receptors are enriched in the microsomal membrane [8, 49]. Although those data suggest that sigma-1 receptors are localized in the endoplasmic reticulum membrane, immunocytochemical studies have just recently reported detailed subcellular distributions of the sigma-1 receptor. Extensive immunoreactivities of sigma-1 receptors have thus been detected in the cytoplasmic region (showing an endoplasmic reticulum distribution pattern) of cell bodies in most cells [1, 2, 11, 19, 20, 29, 55]. These results are further strengthened by the fact that the deduced amino acid sequence of the sigma-1 receptor has a double-arginine endoplasmic reticulum retention signal on the N-terminus [15, 33, 70, 72]. This signal is known to direct the retrieval of membrane proteins from Golgi apparatus to the ER *via* a retrograde transport pathway [69]. Immunofluorescence studies have shown that polyclonal sigma-1 receptor antibodies predominantly stain cytoplasmic areas in neuronal and retinal cells as well [1, 20, 55, 73]. An electron microscopic study of rat brain slices also indicated that the sigma-1 receptor immunostaining was mostly associated with the perikarya and dendrites of neurons [1]. At the level of synaptic contacts, intense immunostaining was associated with the postsynaptic thickening, whereas the presynaptic axons were devoid of immunostaining [1]. These data indicate that the sigma-1 receptor localizes mainly on the endoplasmic reticulum of the cell body and shows a post-synaptic distribution in neurons in the brain. In the primary culture of the rat hippocampus, moderate levels of the sigma-1 receptor are expressed in the perikarya and dendrites of pyramidal neurons [21]. Immunoreactivities in astrocytes are weaker [21]. Significantly higher levels of sigma-1 receptors are expressed in differentiated oligodendrocytes [21] (Fig. (3)). Sigma-1 receptors are also expressed in Schwann cells. Double immunofluorescence studies showed that sigma-1 receptors co-localized with S100 protein, a specific marker

Fig. (2). Brain distribution of sigma-1 receptors.

Sigma-1 receptors in adult rat brains were visualized by immunofluorescence using anti-sigma-1 receptor antibodies and Alexa480-conjugated secondary antibodies. Images were captured by fluorescence confocal microscopy. (A) Olfactory bulb, (B-C) parietal cortex. Arrows indicate dendrites. (D) Subventricular zone. LV: lateral ventricle. (E-F) Hippocampus CA3, (G) corpus callosum, (H-I) cerebellum. Scale bars in A, B, D, and H are 100 μ m, others are 20 μ m.

of Schwann cells, in both rat sciatic nerve Schwann cells and Schwann cells in cultures [61]. Interestingly, it was recently demonstrated that sigma-1 receptors expressed by the gene transfer target plasma membranes of *Xenopus* oocytes and are coupled to Kv1.4 potassium channels [4]. The localization of sigma-1 receptors on the plasma membrane might be possible in particular cell types and/or specialized domains in the plasma membrane, for example being at post-synaptic densities of dendritic spines. Besides, dynamic translocation of sigma-1 receptors may explain the localization of the receptors at the plasma membrane of cells (see 4.-A. ii).

Recently, the sigma-1 receptors' distribution on the endoplasmic reticulum membrane has been examined in greater details. It was found that sigma-1 receptors are present on nuclear envelopes and endoplasmic reticulum reticular networks, but a large portion of sigma-1 receptors target

globular protrusions of endoplasmic reticulum membranes, which are enriched in lipids in NG108 cells [19, 20] (Fig. (3)). Although a bulk of endoplasmic reticulum membranes contains low cholesterol, the endoplasmic reticulum globules are composed of moderate amounts of cholesterol and neutral lipids [20]. C-terminally yellow fluorescence protein (YFP)-tagged sigma-1 receptors exert the same distribution pattern to that of endogenous sigma-1 receptors in NG108 cells [19, 20]. Using the real-time monitoring of confocal microscopy, we demonstrated that YFP-tagged sigma-1 receptors move from the lipid-enriched globules to tubular elements on the endoplasmic reticulum [19]. In primary cells of rat oligodendrocytes, sigma-1 receptors also target the endoplasmic reticulum subcompartments that are extremely enriched in cholesterol and galactosylceramides [21] (Fig. (3)). Orthogonal sectioning by Z-axis confocal microscopy

clearly demonstrated that the sigma-1 receptor-enriched globules are localized between plasma membranes of flat myelin sheets, confirming that the globules are of cytosolic structures [21] (Fig. (3)).

Fig. (3). Cellular distribution of sigma-1 receptors.

Sigma-1 receptors are detected by immunofluorescence as described in Fig. (2). (a) Cellular distribution of sigma-1 receptors in a NG-108 cell. Sigma-1 receptors are highly clustered and localized at the perinuclear region in NG-108 cells. (b) Cellular distribution of sigma-1 receptors in a mature oligodendrocyte from the rat brain. Z-dimensional images are captured along horizontal and vertical white lines, respectively. Orthogonal sections indicate that clusters of sigma-1 receptors are located in cytoplasm of the myelin sheet. Scale bars=10 μ m.

One recent finding on the sigma-1 receptor distribution that should be emphasized here is that sigma-1 receptors form detergent-insoluble lipid microdomains (lipid rafts) on the endoplasmic reticulum membranes [19-21]. Lipids had been originally thought to play merely a passive role as simply building up biological membranes, thus separating the internal milieu from the external environment. In the last decade, a new model has been proposed that predicts the existence of highly clustered lipids microdomains (also called as "lipid rafts") in cellular membranes [2, 75]. Lipid

rafts consist of sphingolipids and cholesterol and are shown to regulate varieties of cellular functions (e.g., protein sorting, clustering scaffold proteins and trophic factor receptors) [2, 75]. The roles of lipid rafts on the plasma membrane have been extensively explored, but little is known about functions of lipid rafts on the endoplasmic reticulum membranes. However, a few recent studies indicate that endoplasmic reticulum lipid rafts may play roles in the segregation of endoplasmic reticulum proteins and in cellular differentiation [21, 56]. It was shown that a portion of sigma-1 receptors exists in lipid rafts at the endoplasmic reticulum membrane and that the association of sigma-1 receptors with lipid rafts is crucial for the protein's function in regulating oligo-dendrocyte differentiation [21].

4. CELLULAR BIOLOGICAL AND PHYSIOLOGICAL ROLES OF SIGMA-1 RECEPTORS (TABLE 2)

The cellular actions of sigma-1 receptors or sigma-1 ligands can be divided into acute and chronic actions. The acute action is seen in immediate responses to administration of ligands in most cases, and can occur in a few minutes after an application of ligands. The chronic action is mainly based on the upregulation of sigma-1 receptor proteins, where upregulation can be achieved by chronic treatments of cells or animals with trophic factors or psychostimulants.

4.A. Acute Action of Sigma-1 Receptor Ligands on Cellular Functions

This section describes acute effects of sigma-1 ligands focusing particularly on their effects on ion-channel activities and translocation of sigma-1 receptors. However, other acute actions of sigma-1 receptors have been reported in a number of literature (analgesia, neuroprotection, etc). Readers are referred to recent comprehensive review articles for details of other acute actions of sigma-1 ligands [3, 13, 22, 42, 82].

4.A.i. Channel Regulation

The effects of sigma-1 receptors and the ligands on ion channels have been most extensively examined. Soriani and colleagues reported that two sigma-1 receptor ligands, JO 1784 and (+)-pentazocine, decrease the transient outward potassium current in cultured frog melanotrope cells by using the patch-clamp recording [77]. The action of (+)-pentazocine was reversible, and was markedly reduced by the sigma-1 receptor antagonist, NE 100. In whole-cell experiments, internal dialysis of guanosine-5'-O-(3-thiophosphate) (100 μ M) irreversibly prolonged the response to (+)-pentazocine [77]. Jackson and colleagues reported the modulation by sigma receptor ligands of K^+ channels in rodent neurohypophysis by using the whole-terminal patch-clamp technique [4, 39, 96]. They found that sigma receptor ligands inhibit K^+ current through two distinct K^+ channels, the A-channel and the Ca^{2+} -dependent K^+ channel [96]. But, in their study, internal perfusion with either guanosine 5'-O-(2-thiodiphosphate) or GTP did not show effects on sigma ligand-induced K^+ current, indicating that signal transduction is independent of G-proteins [39]. They proposed that sigma-1 receptors serve as auxiliary subunits to voltage-gated K^+ channels [4, 39, 96]. A few issues that may be clarified in the

Table 2. Acute or Chronic Actions of Sigma-1 Receptors or Their Ligands

Subject	Pretreatment (time, compounds)	Type of action
Acute action		
K ⁺ channel [4, 39, 77, 96]	<5 min, sigma-1 ligands	Inhibition (modulatory)
Ca ²⁺ channel [17, 99]	3-10 min, sigma-1 ligands	Inhibition (modulatory)
NMDA receptors [16, 31, 53]	>3 min, sigma-1 ligands	Inhibition (modulatory)
Glutamate- induced Ca ²⁺ mobilization [54]	<1 min, sigma-1 ligands	Potentialiation (modulatory)
IP3 receptor or other Ca ²⁺ release from the ER [17, 18, 26, 57, 62, 92]	>10 min, sigma-1 ligands and neurosteroids	Potentialiation (modulatory)
Anti-amnesic actions in behavioral models [45, 48]	<60 min, sigma-1 agonists	Improvement of amnesia by agonists (modulatory)
Translocation [18, 19]	>10 min or hours Sigma-1 agonists	Ligands <i>per se</i> initiate translocation (not modulatory)
Chronic action		
Antipsychotic action of neuroleptics [28]	2 weeks, Haloperidol 4mg/kg/day i.p.	Down-regulation of sigma-1 receptors in rat brains
Behavioral sensitization [27]	10 days, METH 4mg/kg/day i.p.	Up-regulation of sigma-1 receptors in rat brains
Drug self-administration [78]	5 weeks, METH self-administration approx. 1.2 mg/kg/day, i.v.	Up-regulation of sigma-1 receptors in rat brains
Cellular differentiation [21, 85]	1-3 day, NGF, no sigma-1 ligands	Up-regulation of sigma-1 receptors & potentialiation of differentiation in PC-12 cells
	2 days, N1-supplement**, no sigma-1 ligand	Up-regulation of sigma-1 receptors & potentialiation of myelination
Cholesterol redistribution inside cells [20, 23, 86]	2 days, no sigma-1 ligand, sigma-1 receptor overexpression	Increase in cholesterol in lipid rafts
Reconstitution of gangliosides in lipid rafts [23, 86]	2 days, no sigma-1 ligands, sigma-1 receptor overexpression	Increase in GD1a ganglioside in lipid rafts

"Modulatory": Ligands *per se* have no effect, but exert modulatory actions when channels or certain physiological pathways are activated. METH: methamphetamine. Up- or down-regulation indicates protein levels of sigma-1 receptors. ** See reference [21] for components.

future include 1) why micromolar concentrations of sigma ligands are required for the inhibition of K⁺ current, and 2) why the agonist-antagonist relationship of sigma ligands is missing in some studies?

The sigma-1 receptor's modulation of NMDA receptor-coupled ion channels has been also demonstrated. The preincubation of primary neurons with sigma-1 receptor ligands attenuated NMDA-induced Ca²⁺ mobilization [16]. The potency of ligands inhibiting the NMDA-induced Ca²⁺ influx correlates with their binding affinity orders at sigma-1 receptors [16]. However, micromolar concentrations of ligands were required for the inhibition. Further, no agonist-antagonist relationship could be observed in the study, perhaps due to the use of non-selective sigma antagonists [16]. A clear agonist-antagonist relationship has been demonstrated in later studies using MS-377 ((R-(+)-1-(4-chlorophenyl)-3-[4-(2-methoxyethyl)piperazin-1-yl]methyl-2-pyrrolidinone L-tartrate) and NE-100, newly synthesized

selective sigma-1 receptor antagonists without an affinity for the NMDA receptor ion-channel complex [31, 54]. MS-377 does not affect the basal Ca²⁺ concentration and the NMDA-induced Ca²⁺ influx by itself, whereas significantly blocks the reduction by SKF-10047 of the NMDA-induced Ca²⁺ influx [31]. This finding suggests an indirect modulatory pathway on the NMDA receptor *via* the activation of sigma-1 receptors. Another study successfully demonstrates an agonist-antagonist relation in regulation of glutamate receptors by sigma-1 ligands [54]. One-min pulses of (+)-benzomorphans or JO-1784 concomitantly with glutamate were shown to potentiate Ca²⁺ mobilizations in primary culture of neurons. The potentiation was prevented by a selective sigma-1 antagonist NE-100 [54]. On the other hand, the longer preincubation with sigma ligands (i.e., 10 min) causes the inhibition of NMDA channels [54]. This finding indicates that sigma-1 ligands might exert different effects on the NMDA ion channel depending on their preincubation time.

Several studies reported that the sigma-1 receptor modulates the neuronal firing and the neurotransmitter release. Monnet *et al.* found that sigma-1 ligands, although exhibiting no effect by themselves, enhance the NMDA-induced, but not the kainate-induced neuronal firing in the CA3 region of rat hippocampal slices [53]. Sigma-1 receptor ligands were shown to modulate the depolarization- or the NMDA-induced dopamine release from brain slices [58]. Nuwayhid and Werling recently demonstrated that sigma-1 receptor agonists regulate the NMDA-induced dopamine release from rat striatal slices *via* protein kinase C [58]. Debonnel and colleagues have examined effects of sigma-1 receptor ligands on neuronal activities in different brain areas of anesthetized animals [5]. They demonstrated that sigma-1 ligands modulate neuronal activities in both glutamatergic and serotonergic neurons [5].

Sigma receptors affect Ca^{2+} channels in neurons and a cancer cell line. In the whole cell patch-clamp studies, sigma ligands were shown to rapidly depress peak calcium channel currents in a reversible manner in both superior cervical ganglion and intracardiac ganglion neurons, very likely *via* sigma-2 receptors [99]. In NG108 cells, inhibition or potentiation of depolarization-induced Ca^{2+} influx by sigma ligands is sigma-1 receptor antisense-sensitive, suggesting the involvement of sigma-1 receptors in the regulation of Ca^{2+} channels in this cell type [17]. More experiments combining electrophysiological techniques and simple artificial membrane systems like liposomes may be required to confirm functional and physical interactions between sigma-1 receptors and calcium channels.

Several studies demonstrated that both type-1 and -2 sigma receptors regulate Ca^{2+} efflux from the endoplasmic reticulum [17, 18, 26, 57, 62, 92]. Novakova *et al.* reported that BD-737 (1-100 nM) and BD-1047 (10-100 nM) cause an increase in the intracellular concentration of inositol 1,4,5-trisphosphate (IP3) and Ca^{2+} concentrations in cardiac myocytes [57]. BD-1047 gradually increases the basal Ca^{2+} concentration to 4.4 folds 5 min after the application. Using human neuroblastoma [57], Vilner and Bowen confirmed that BD737 induces intracellular Ca^{2+} mobilization *via* sigma-2 receptors. They found that the effect of BD737 involves two modes of action; a transient release of Ca^{2+} from the endoplasmic reticulum and a sustained rise in $[\text{Ca}^{2+}]_i$ derived from thapsigargin-insensitive intracellular Ca^{2+} stores [92].

The type-1 sigma receptor has been reported to modulate Ca^{2+} signaling *via* IP3 receptors on the endoplasmic reticulum [17, 18, 26, 62]. Sigma-1 receptor agonists potentiate IP3-induced Ca^{2+} mobilization in a biphasic dose-dependent manner in a neuronal cell line [17, 26]. The potentiation is blocked by sigma-1 receptor antagonists or by transfection of the sigma-1 receptor antisense oligodeoxynucleotides [17]. In human neuroblastoma, the preincubation of low micromolar concentrations of neurosteroids pregnenolone and dehydroepiandrosterone (DHEA), as well as sigma-1 receptor agonist (+)pentazocine, potentiate the IP3-induced increase of $[\text{Ca}^{2+}]_i$, suggesting that these neurosteroids are sigma-1 receptor agonists [26]. The regulation of IP3 receptors by sigma-1 receptors appears to be mediated, at least in part, by the association of sigma-1 receptors with

cytoskeletal protein ankyrin on the endoplasmic reticulum [18]. Ankyrin is known to bind IP3 receptors and inhibit the Ca^{2+} release *via* IP3 receptors [52]. A recent study suggests that sigma-1 receptors regulate the interaction between ankyrin and IP3 receptors [18]. Localization of sigma-1 receptors on the endoplasmic reticulum is in perfect agreement with sigma-1 receptors regulating Ca^{2+} signaling *via* IP3 receptors on the endoplasmic reticulum. Regarding the regulation of IP3 channels by sigma-1 receptor ligands, the longer incubation time (>10 min) is usually required [17, 26] when compared to that required for the inhibition of K^+ channels [39, 96], suggesting an uptake of ligands to reach the endoplasmic reticulum sigma-1 receptor sites. The finding that sigma-1 ligands do not affect the IP3 formation in NG108 cells further supports that the regulation of IP3 receptors by sigma-1 receptors occurs, at least in part, at the endoplasmic reticulum level [17].

4.A.ii. Translocation

The molecular mechanism of acute effects caused by sigma-1 ligands is not fully clarified at present. In almost all studies mentioned above, actions of sigma-1 ligands are typically modulatory; they show no pharmacological action *per se*, but exhibit modulatory actions when K^+ channels, NMDA receptors or IP3 receptors are activated (See Table 2). However, recent studies demonstrated that acute administration of sigma-1 ligands *per se* can cause a peculiar biochemical action; that is, the sigma-1 receptor translocation [17-19, 55]. Translocation of sigma-1 receptors requires merely sigma-1 ligands, but not activation of other receptors and channels. Thus, this direct biochemical response to sigma-1 ligands may be one of the mechanisms underlying acute actions of ligands.

Sigma-1 receptors are shown to translocate from the endoplasmic reticulum to peripheries of cells upon the application of sigma-1 ligands. Morin-Surun *et al.* (1999) first reported the translocation of sigma-1 receptors [55]. They found that the perfusion of (+)pentazocine through the basilar artery causes a shift of sigma-1 receptors from cytoplasmic areas toward the plasma membrane in the brain stem area of guinea pigs [55]. This result suggests that intracellular sigma-1 receptors can regulate components in plasma membrane-bound signal transduction moieties (e.g., PKC) *via* the translocation. It was also found that sigma-1 receptors translocate in NG108 cells and regulate endoplasmic reticulum molecules implicated in intracellular signal transduction [17, 18]. It was found that a portion of sigma-1 receptors on the endoplasmic reticulum is associated with ankyrin, a cytoskeletal adaptor protein that inhibits Ca^{2+} release through IP3 receptors on the endoplasmic reticulum [18]. Sigma-1 receptor agonists cause the dissociation and translocation of sigma-1 receptor and ankyrin as a complex from IP3 receptors and, as a resultant, potentiate the Ca^{2+} release through IP3 receptors [28, 81]. The subcellular fractionation studies and the real-time monitoring of YFP-tagged sigma-1 receptors in living cells further confirmed that sigma-1 ligands induce the translocation of sigma-1 receptors. Sigma-1 agonists can shift sigma-1 receptors from the low-density P3 fraction to P1 and P2 fractions when given to the cell for a period of 10 min [18, 19]. In living cell

imaging, YFP-tagged sigma-1 receptors move out lipid-enriched endoplasmic reticulum globules and slide on the endoplasmic reticulum reticular network toward the nuclear membrane and the plasma membrane in NG108 cells [19]. In the neurite-like process, YFP-tagged sigma-1 receptors move anterogradely from the cell body to the tip of the process [19]. Interestingly, this translocation induced by sigma-1 ligands could be seen even more intensely when the culture medium containing fetal calf serum was replaced by the balanced salt solution in our test system [19]. This latter finding indicates that there might be endogenous factor(s) that may regulate the ligand-induced translocation. The factor(s) are not identified at present.

4.B. Chronic Action of Sigma-1 Receptors: Receptor Up-Regulation

4.B.i. Sigma-1 Receptor Up- or Down-Regulation in *In Vivo*

In early binding studies, psychotropic drugs were demonstrated to up- or down regulate sigma-1 receptors following chronic treatments [27, 28]. The effect of repeated exposures of rats to haloperidol (4 mg/kg/day for 14 days) on levels of sigma-1 binding sites was first investigated. Repeated administrations of haloperidol induced a dramatic down regulation of (+)[³H]-3-PPP binding sites (75% decrease in the number of binding sites compared to controls), which lasted for at least 7 days after the termination of haloperidol [28]. However, on 28th day after termination of the haloperidol-treatment, the total number of (+)[³H]-3-PPP binding sites was recovered [28]. Importantly, the same treatment did not alter sigma-2 sites labeled by [³H]DTG [28]. Furthermore, Itzhak *et al.* reported that a significant up regulation (130-145% of control B_{max}) of sigma receptors, labeled with [³H](+)-pentazocine, is observed in the substantia nigra, frontal cortex and cerebellum of rats treated with methamphetamine (4.0 mg/kg per day; 10 days) [27]. An upregulation of sigma-1 receptors was also found in the brain of rats self-administering methamphetamine [78]. In this experiment, protein levels of sigma-1 receptors were assessed by Western blotting with specific antibodies against the rat sigma-1 receptor [78]. Sigma-1 receptors were upregulated in the midbrain of rats that self-administered methamphetamine, but not in those that received passive injections of methamphetamine in a noncontingent manner (yoked control) [78]. Because doses of methamphetamine received by animals in the experiment are very low, and because the yoked-controlled group receiving the same dose of methamphetamine as self-administering rats showed no change in sigma-1 receptors, methamphetamine may not cause an upregulation of sigma-1 receptors through the direct binding to sigma-1 receptors (K_i of methamphetamine for sigma-1 receptors = 2 μM) in this behavioral paradigm. Therefore, it was speculated that signal transductions downstream of dopamine receptors, possibly *via* a cAMP-dependent pathway, might be involved in the regulation of sigma-1 receptor expression [78].

4.B.ii. Sigma-1 Receptor Upregulation in *In Vitro*

The upregulation of sigma-1 receptors have also been demonstrated *in vitro*. NGF is well known to induce

neuronal differentiation in rat pheochromocytoma PC-12 cells [85]. It was found that the treatment of PC-12 cells with NGF (1-3 days) causes an upregulation of sigma-1 receptors [85]. This effect of NGF was further potentiated by the co-administration of NGF with sigma-1 receptor agonists [85]. Importantly, the inhibition of the sigma-1 receptor upregulation by antisense oligodeoxynucleotides blocks NGF-induced neuritogenesis in PC-12 cells, indicating that upregulation of sigma-1 receptors is crucial for the NGF action in promoting cellular differentiation [85]. Dibutylic cAMP, a well-known inducer of differentiation, causes upregulation of sigma-1 receptors and cellular differentiation in NG108 cells [78]. It is also reported that sigma-1 receptors are upregulated during the maturation of oligodendrocytes [21]. Oligodendrocyte progenitors express low levels of sigma-1 receptors [21]. However, once the differentiation process is initiated, sigma-1 receptors begin to increase [21]. The similar expression pattern of sigma-1 receptors in oligodendrocytes can be found in rat brains [21].

Taken together, these data demonstrated that up- or down regulation of sigma-1 receptors in the brain occurs as a physiological response to certain psychotropic drugs or activation of trophic factor receptors. The importance of the upregulation of sigma-1 receptors will be discussed in the next section.

4.C. Impact of Sigma-1 Receptor Upregulation on Cellular Functions

4.C.i. Lipid Transport and Membrane Remodeling

As mentioned above, sigma-1 receptors target lipid-enriched domains on the endoplasmic reticulum. The endoplasmic reticulum is an organelle, which synthesizes most cellular lipids including cholesterol, neutral lipids, fatty acids and ceramides. However, it is not fully understood how synthesized lipids are transported from the endoplasmic reticulum to other cellular membrane *loci*. It was recently found that the overexpression of sigma-1 receptors increases cholesterol contents in the Golgi and in the plasma membrane lipid rafts in NG108 cells as well as in PC-12 cells [23, 86]. On the contrary, when functionally dominant negative sigma-1 receptors are transfected, a large amount of neutral lipids and cholesterol are retained in the endoplasmic reticulum, causing the pathological aggregation of the endoplasmic reticulum [20]. In mutant-transfected cells, neutral lipids cannot be transported to cytosolic lipid droplets and cholesterol in the Golgi and plasma membrane is concomitantly decreased [20]. Functionally dominant negative sigma-1 receptors also decrease the level of lipid rafts on the plasma membrane [19, 20]. These observations indicate that one potential function of sigma-1 receptors on the endoplasmic reticulum is to regulate the compartmentalization and transport of lipids at endoplasmic reticulum [20]. By regulating the lipid transport in this fashion, sigma-1 receptors might indirectly modulate the formation of plasma membrane lipid rafts. Importantly, sigma-1 receptor overexpression also alters glycosphingolipid components in lipid rafts [86]. Sigma-1 receptor overexpression increases GD1a ganglioside, a highly glycosylated ganglioside, but decreases GM1 and GM2 in lipid rafts in PC-12 cells [86]. Therefore, it was proposed that sigma-1 receptors do not merely

regulate the number of lipid rafts, but also cause reconstitution of lipid constituents in cellular membranes [24].

4.C.ii. Cellular Differentiation and Myelination

One important role of the sigma-1 receptor upregulation is to regulate cellular differentiation. Sigma-1 receptor agonists enhance the differentiation (e.g., neurite sprouting) of PC-12 cells caused by NGF [85]. A sigma-1 receptor antagonist, NE-100, antagonized only the enhancement of the NGF action by sigma-1 receptor agonists [85]. Although the agonist-antagonist relationship of sigma-1 ligands can be observed in these experiments, it was concluded that the modulation of the neurite formation by sigma-1 receptors is mediated *via* upregulation of sigma-1 receptors based on the following findings: 1) sigma-1 agonists potentiate NGF-induced upregulation of sigma-1 receptors and NGF-induced differentiation, 2) antisense oligodeoxynucleotides against sigma-1 receptors block NGF-induced differentiation, and 3) overexpression of sigma-1 receptors by transfecting cDNA *per se* potentiates NGF-induced differentiation [85]. Interestingly, EGF also caused neurite sprouting in sigma-1 receptor-overexpressing PC-12 cells, although in wild-type PC-12 cells, EGF promotes only the proliferation [86]. While sigma-1 receptor overexpression did not change EGF receptor levels in PC-12 cells, the overexpression decreased the level of EGF receptors in lipid rafts and concomitantly potentiated the EGF-induced ERK MAP kinase activity [86]. It was therefore proposed that the potentiation of the EGF-induced MAP kinase activity might be related to the reconstitution of lipid constituents in lipid rafts due to the sigma-1 receptor overexpression [24].

The importance of sigma-1 receptor upregulation in cellular differentiation was further examined in the rat brain and in primary cells [21]. In the brain, sigma-1 receptors are expressed in neurons and in oligodendrocytes respectively, at a moderate and a high level [21]. In rat oligodendrocyte progenitors, levels of sigma-1 receptors, especially those residing in lipid rafts, increase as cells differentiate [21]. Sigma-1 receptors also increase rapidly in oligodendrocytes and myelin of developing rat brains 2 weeks after birth [21]. Overexpression of sigma-1 receptors enhances the differentiation of oligodendrocyte progenitors, whereas knockdown of sigma-1 receptors by siRNA inhibits the differentiation, indicating that upregulation of sigma-1 receptors regulate the differentiation of oligodendrocytes and myelin formation [21].

The involvement of sigma-1 receptors in myelination was also reported by Demerens *et al.* [10]. They tested the potential of eliprodil, a neuroprotective agent with a high affinity for sigma-1 receptors, in promoting myelination in neuron-oligodendrocyte cocultures [10]. They found that 1 μ M of eliprodil does not change the number of either neurons or oligodendrocytes, however, it induces an increase in myelination [10]. These findings, when taken together, suggest that sigma-1 receptor ligands may be of therapeutic interest in demyelinating diseases such as multiple sclerosis [21, 10].

Although the upregulation of sigma-1 receptors seems to be crucial for the differentiation in certain types of cells, it is

still unknown how sigma-1 receptor upregulation affects cellular differentiation. During differentiation, cells might need a dynamic shift in lipid metabolism/transport or in ion channel activities that is regulated by sigma-1 receptors [4, 20, 23]. The overexpression studies clearly indicate that the upregulation of sigma-1 receptors *per se*, even without exogenous ligands, appear to be sufficient to promote the potentiation of NGF-induced neuronal differentiation, oligodendrocyte differentiation and myelination, and the reconstitution of lipid rafts [21, 85, 86]. These facts suggest that the sigma-1 receptor may possess an innate biological activity [22]. Existence of endogenous sigma-1 receptor ligands may not explain the already existing activity of sigma-1 receptors because sigma-1 receptor antagonists *per se*, which are supposed to replace endogenous ligand, lack pharmacological effects in those experiments. The ligands may work as modulators of the innate biological activity (see [22]).

5. BEHAVIORAL ROLES OF THE SIGMA-1 RECEPTOR AND ITS LIGANDS

Sigma-1 receptor ligands have been tested in a variety of behavioral models which include learning and memory, psychosis, depression, and drug abuse. In this review article, the focus is psychosis and drug abuse models only. For results in other models, please see the recent comprehensive review articles [13, 22, 42, 46, 48, 76].

The demonstrations of several antipsychotics and cocaine possessing high to moderate affinities for sigma-1 receptors in radioligand binding assays inspired researchers to test sigma-1 receptor ligands in animal models of schizophrenia and drug dependence [42, 74]. Several sigma-1 receptor antagonists have been synthesized with the expectation that they may represent a new class of antipsychotics. Pharmacological effects of these ligands were assessed typically by applying the ligands to amphetamine- or PCP-treated animals. Putative sigma-1 receptor antagonists such as BMY-14802 (alpha-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine butanol monohydrochloride) were shown to inhibit the amphetamine-induced locomotor activity and apomorphine-induced stereotypy [59]. Recently synthesized more selective sigma-1 antagonists, however, could not replicate those results. Both NE-100 and MS-377, selective sigma-1 receptor antagonists, do not affect locomotion activity induced by acute injections of amphetamine [59, 83, 84]. The discrepancy seen in these psychostimulant-induced locomotion studies has not been solved yet. Because only high doses (>20 mg/kg) of BMY-14802 and rimcazone were used in some experiments, the inhibition of psychostimulant-induced locomotor activity may not be solely due to the blockade of sigma-1 receptors. Some other factors, such as drug metabolism and actions of their metabolites, should be also considered when interpreting the results. A recent study using sigma receptor antagonist LR172 raises one possibility that sigma-2 receptors might be involved in the inhibition of cocaine-induced locomotor activity caused by sigma receptor ligands [50].

NE-100 is the most well-defined selective sigma-1 receptor antagonist [59]. As mentioned above, NE-100 does not affect the behavior induced by amphetamine, but can

inhibit PCP-induced behavior alterations [59]. The order of the inhibitory potencies of the PCP-induced head weaving by sigma receptor antagonists (NE-100>haloperidol>BMY 14802>Dup 734) correlated well with their affinities for sigma-1 receptors, but correlated poorly with their affinities for dopamine D2 receptors [59]. On the other hand, selective sigma-1 receptor agonists such as (+)pentazocine enhanced the psychotomimetic effect of MK-801, and this enhancement was blocked by NE-100 [59]. On the contrary, in animal models of amnesia, sigma-1 agonists have been shown to improve MK-801-induced memory deficits [47]. These findings suggest that sigma-1 receptors might modulate glutamatergic neurons by affecting functions of the NMDA receptor, although the exact mechanism is unclear [34]. A number of recent studies suggest that glutamatergic neurons play important roles in pathophysiology of schizophrenia and some psychological components of drug dependence, such as drug seeking and craving [30, 88]. Selective sigma-1 antagonists might represent unique psychotropic drugs that can modulate glutamatergic system in the brain.

So far, cocaine is the most well-examined drug of abuse in its relation to the sigma-1 receptor. One of the reasons is that cocaine possesses a moderate affinity for sigma-1 receptors in radioligand binding assays (2-4 μ M Ki) [42, 74]. Matsumoto and colleagues found that sigma receptor antagonists (1 mg/kg) significantly inhibit convulsion and lethality induced by toxic doses of cocaine [41, 43]. The toxicity of cocaine was potentiated by sigma-1 receptor agonists [41, 43]. This finding indicates that the actions of cocaine, at least in part, might be mediated by its binding to sigma-1 receptors. However, sigma-1 receptors are endoplasmic reticulum proteins and the affinity of cocaine at sigma-1 receptors, at least in its salt form, is in the μ M range. It remains unknown at present whether cocaine can penetrate plasma membrane in sufficient concentrations to act on the sigma-1 receptors in brains of animals or humans that self-administer cocaine. Cocaine is a psychostimulant whose action involves a transport blockade of dopamine reuptake and consequently over-activating post-synaptic dopamine receptors [30]. Therefore, effects of sigma-1 receptor ligands in cocaine-induced behaviors might also be exerted at the downstream of dopamine receptors. This possibility was first addressed by Maurice and colleagues. In the study, they employed the cocaine-induced conditioned place preference (CPP) to assess effects of sigma-1 ligands [68]. CPP is a routine test to measure preference of animals to certain drugs. They found that a sigma-1 receptor antagonist or injections of sigma-1 receptor antisense oligodeoxynucleotides (i.c.v) attenuate cocaine-induced CPP in mice [67]. Importantly, the sigma-1 agonist alone did not cause a CPP in this study. They next tested sigma-1 receptor antagonists on CPP induced by another dopamine transporter inhibitor BTCP (N-[1-(2-benzo(b)thiophenyl)cyclohexyl]piperidine), which has no sigma receptor affinity. Interestingly, BTCP-induced CPP was also blocked by a sigma-1 receptor antagonist [68]. These results support the notion that sigma-1 receptors are involved in the downstream signaling of dopaminergic neurotransmission in the CPP model. This finding leads us to further speculate that sigma-1 receptor ligands might affect actions of other drugs of dependence possessing no affinities for sigma-1 receptors,

but activating the dopaminergic system (e.g., morphine, alcohol, nicotine). To further consolidate this interesting hypothesis, future experiments should address the followings; 1) Does the sigma-1 receptor regulation occur at the intracellular level in dopamine neurons (such as the regulation of the dopamine release)?, or 2) Does the regulation occur at the network level of neurons (Dopaminergic neurons may terminate at sigma-1 receptor-regulated neurons)?

A few studies examined possible involvements of sigma-1 receptors in the action of chronic psychostimulants. It is well-established that chronic administrations of psychostimulants augment the sensitivity of animals (even humans) to psychostimulants. Thus, after chronic injections of cocaine or methamphetamine (for 7-14 days), the same dose or even lower doses of psychostimulants can induce a higher magnitude of behavioral alterations (so-called behavioral sensitization). Ujike *et al.* reported that putative sigma receptor antagonists, at doses *per se* not affecting spontaneous locomotor activity, block the development of behavioral sensitization induced by cocaine and methamphetamine [89, 90]. Using a more selective sigma-1 antagonist, MS-377, another research group was able to replicate Ujike's finding [83]. Methamphetamine-induced behavioral sensitization in rats was blocked by MS-377 [83]. The finding of the sigma-1 receptor upregulation in rat brains after chronic methamphetamine (10 days) [27] suggests that the action of sigma-1 receptor ligands in the sensitization model may be related to their "chronic action" rather than their "acute actions" (see "Chronic action of sigma-1 receptors: receptor up-regulation"). Roles of sigma-1 receptors were also examined in chronic methamphetamine self-administering rats [78]. Sigma-1 receptors are upregulated in the midbrain of rats that self-administered methamphetamine, but not in those that received passive injections of methamphetamine in a noncontingent manner (yoked controls) [78]. Sigma-1 receptors might play a role in neuroadaptation to drug-induced reward processes. However, further studies are required to confirm whether sigma-1 receptor upregulation plays a causal role in the development of drug-induced reward process.

6. KNOCKOUT MOUSE

The sigma-1 receptor knockout mouse was generated in 2003 [36]. Using the sigma-1 receptor ligand [³H](+)-pentazocine as a radioligand, no binding activity in brain membranes was observed in sigma-1 receptor (-/-) mice [36]. Importantly, binding affinity experiments using [³H]DTG, a sigma-1 and -2 receptor ligand, revealed the presence of the sigma-2 receptor binding sites (*B* max, 310 fmol/mg protein), confirming that the sigma-2 receptor is encoded by a different gene and may not be a product of an alternative splicing variant of the sigma-1 receptor gene.

The deletion of sigma-1 receptors in mice is not lethal. Mutant (-/-) mice gain body weight at a rate similar to that of the wild type mice [36]. However, more details should be examined if the knockout of sigma-1 receptors may affect development. Acute knockdown of sigma-1 receptors can cause a number of abnormalities in cellular functions *in vitro* [21]. Thus, compensatory mechanisms may have kicked in knockout mice.

Nevertheless, some interesting findings were observed in behavioral experiments using sigma-1 receptor (-/-) mice. A study of the spontaneous activity revealed that the mean mobile time for mutant (-/-) mice was significantly lower than the spontaneous locomotor activity of wild-type mice [36]. In mutant (-/-) mice, (+)SKF10047-induced locomotor activities are decreased when compared to wild type mice, suggesting that the (+)SKF10047-induced psychotomimetic action is, at least in part, mediated *via* sigma-1 receptors [36]. Further studies using sigma-1 receptor knockout mice will surely help understand the molecular mechanisms of sigma-1 receptors at least in certain behavioral paradigms.

7. HUMAN STUDY

Because early studies suggested an implication of sigma-1 receptors in schizophrenia, several sigma ligands have been developed as antipsychotics and some of them were introduced in clinical trials. So far, an apparent antipsychotic action of sigma ligands in human has not been confirmed. Recent studies suggest usefulness of sigma-1 ligands for negative symptoms of schizophrenia, depression, and dementia. Please refer to recent reviews for details in human clinical trials [22, 93].

A recent new attempt is to introduce sigma-1 receptor ligands in PET scanning. A few groups are evaluating radio-labeled sigma-1 ligands in non-human primates. [¹⁸F]FPS ([¹⁸F]1-(Fluoropropyl)-4-[(4-cyanophenoxy)methyl]piperidine) and [¹¹C]SA4503 (1-[(4-methoxy-¹¹C]-3,4-dimethoxy-phenethyl)-4-(3-phenylpropyl)piperazine) were tested in primates and favorable results were obtained in assessing the drugs' distribution and safety [32, 95]. They are expected to be introduced in human PET studies in the near future.

PERSPECTIVES

In the last decade, impressive progress has been made in our understanding of sigma receptors. Specifically, the cloning of the sigma-1 receptor cDNA greatly contributes the understanding. Important progresses also include 1) identifying sigma-1 receptor binding sites by pharmacological and molecular approaches, 2) clarifying details of cellular and subcellular distributions of sigma-1 receptors, and 3) providing implications of sigma-1 receptors in certain behavioral models and human diseases. The *in vitro* and *in vivo* studies implicate sigma-1 receptors in neuronal membrane remodeling and neuronal plasticity. The over-expression studies strongly suggest that the sigma-1 receptor possesses as yet unidentified innate biological activity [22]. The sigma-1 receptor ligands appear to be modulators of the innate biological activity [22]. In analogy, upregulation of sigma-1 receptors may be of the crucial importance in their functioning in the brain. Future projects are expected to define the innate biomolecular function of the sigma-1 receptor that may reconcile diverse actions of sigma-1 receptors and the multi-drug binding property of the protein.

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