# Understanding the Molecular Mechanism of Sigma-1 Receptors: Towards A Hypothesis that Sigma-1 Receptors are Intracellular Amplifiers for Signal Transduction

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**Abstract:** Although sigma receptors were discovered in 1982, the biochemical and physiological roles of sigma receptors have just begun to unveil. Sigma receptors are non-opioid, non-phencyclidine receptors that contain two subtypes: sigma-1 and sigma-2 receptors. The sigma-1 receptor has been cloned and its sequence does not resemble that of any mammalian protein. Sigma-2 receptors have not been cloned. The focus of this review will be on sigma-1 receptors. Sigma-1 receptors contain 223 amino acids and reside primarily at the endoplasmic reticulum. Sigma-1 receptors exist mainly in the central nervous system, but also in the periphery. Sigma-1 receptor ligands include cocaine, (+)-benzomorphans like (+)-pentazocine and (+)N-allyl-normetazocine (or (+)-SKF-10047), and endogenous neurosteroids like progesterone and pregnenolone sulfate. Many pharmacological and physiological actions have been attributed to sigma-1 receptors. These include the regulation of IP3 receptors and calcium signaling at the endoplasmic reticulum, mobilization of cytoskeletal adaptor proteins, modulation of nerve growth factor-induced neurite sprouting, modulation of neurotransmitter release and neuronal firing, modulation of potassium channels as a regulatory subunit, alteration of psychostimulant-induced gene expression, and blockade of spreading depression. Behaviorally, sigma-1 receptors are involved in learning and memory, psychostimulant-induced sensitization, cocaine-induced conditioned place preference, and pain perception. Notably, in almost all the aforementioned biochemical and behavioral tests, sigma-1 agonists, while having no effects by themselves, caused the amplification of signal transductions incurred upon the stimulation of the glutamatergic, dopaminergic, IP3-related metabotropic, or nerve growth factor-related systems. Thus, it is hypothesized that sigma-1 receptors, at least in part, are intracellular amplifiers creating a supersensitized state for signal transduction in the biological system.

## HISTORY AND INTRODUCTION

Originally intending to identify a subtype of opioid receptors, the sigma/opioid receptors as proposed by Martin *et al.*. [1], Su [2] used the tritiated prototypic sigma/opioid receptor ligand SKF-10047 (N-allyl-normetazocine) in binding assays and identified a protein that has a nanomolar affinity for SKF-10047. Surprisingly though, the protein had no affinity for the opioid antagonist naloxone. This raised a possibility that the protein identified by Su [2] may not be the sigma/opioid receptor proposed by Martin *et al.*. [1] but may represent a new receptor at which SKF-10047 has an affinity. The protein identified by Su [2] was later termed "sigma receptor", with the word "opioid" dropped to differentiate it from the sigma/opioid receptor and the word "sigma" retained for it being identified with the labeled ligand *S*KF-10047. So far, the sigma/opioid receptor proposed by Martin *et al.*. has not been clearly identified.

The sigma receptor has several peculiarities in its ligand binding profile and its distribution. Firstly, the affinity of dextrorotatory benzomorphans like (+)SKF-10047 is higher than its levorotatory isomer [2]. So is the affinity of (+)pentazocine higher than the (-)pentazocine, and that of (+)cyclazocine much higher than (-)cyclazocine [2]. This stereospecificity is opposite to that seen with all opioid receptor subtypes in either binding assays or behavioral tests. The stereospecificity that the sigma receptor possesses further indicates that the sigma receptor is not an opioid receptor. Secondly, the sigma receptor binds diverse classes of pharmacological agents. In addition to benzomorphans, the sigma receptor ligands include haloperidol, imipramine, fluovoxamine, pimozide, chlorpromazine, dextromethorphan, propranolol, and phencyclidine (see [2]; in descending order of affinity). However, it is important to point out that although haloperidol is the most potent ligand at sigma receptors, as so reported in 1982, other potent dopamine D2 receptor ligands such as sulpiride and spiroperidol have no affinity for sigma receptors [2-6]. Thirdly, neurosteroids like sex hormones progesterone, testosterone, and pregnenolone sulfate have moderate affinity at sigma receptors [7]. Fourthly, in addition to the central nervous system, sigma receptors apparently exist in the periphery, at least as indicated from the binding assays [8,9]. Because phencyclidine has an appreciable affinity for sigma receptors, sigma receptors were confused as the phencyclidine receptors for a period of time. Now it is clear that sigma receptors are not the NMDA(N-methyl-D-aspartate)/phencyclidine receptors

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0929-8673/03 $41.00+.00 

[10]. In addition to using tritiated SKF-10047, sigma receptors have been identified by using tritiated 1,3-di(2-tolyl)-guanidine (DTG) and (+)3-PPP (3-hydroxyphenyl-N-(1-propyl)-piperidine) [4,5].

Because (+)-pentazocine was the second most potent sigma receptor ligand in the original study [2], the binding of (+)pentazocine was further examined by Bowen and colleagues [11]. They discovered that from the binding assays, sigma receptors could be subdivided into two subtypes: sigma-1 receptors and sigma-2 receptors [10-12]. Basically, the ligand binding profile of sigma-1 receptors are the same as the sigma receptor originally described by Su [2] in that dextrorotatory benzomorphans are at least 5-10 fold more potent than their counterpart levorotatory isomers. On the contrary, in sigma-2 receptor binding assays, the levorotatory benzomorphans are more potent compared to their counterpart dextrorotatory isomers [10,11]. Since the discovery of sigma-1 and sigma-2 receptors, many respective selective ligands for each receptor have been described [e.g., 13,14]. So far, (+)pentazocine remains the mostly-used selective ligand for sigma-1 receptors, and [$^3$H](+)-pentazocine has been developed and widely used in radioligand binding studies of sigma-1 receptors [13]. Neurosteroids apparently are selective for sigma-1 receptors [15]. Sigma-1 receptors and sigma-2 receptors have different distribution patterns in the brain [16]. The ontogenesis of the two receptors is also different [15].

The sigma-1 receptor was cloned in 1996 [17]. The ligand binding profile of the cloned sigma-1 receptors are in perfect agreement with that obtained from the sigma-1 receptor binding assays using brain homogenates. Specifically, the stereospecificity of benzomorphan rotatory isomers seen with the cloned sigma-1 receptors is in good agreement with that seen with the sigma-1 receptors binding assays using brain homogenates [17]. Successful clonings of sigma-1 receptors from rat, mouse, human tissues have since been reported [18-21]. The sigma-1 receptor has 223 amino acids with three hydrophobic regions with the middle one being a potential transmembrane segment. It has an endoplasmic reticulum retention signal at the N-terminus [17]. The sequence of sigma-1 receptors does not resemble that of any mammalian proteins [17]. It has a 30.3% identity and 66.4% homology to a fungal. C8-C7 sterol isomerase [17]. Sigma-1 receptors, however, do not possess the sterol isomerase activity [17]. In our own analysis, we noted that at least 5% of the identical amino acids between the mammalian sigma-1 receptor and the yeast C8-C7 isomerase are in the second hydrophobic region which was purported to be the transmembrane domain. This domain has been identified as the sterol-binding domain in the yeast C8-C7 isomerase. Thus, taken together, these observations may explain the fact that although sigma-1 receptors do not have the C8-C7 isomerase activity, sigma-1 receptors can bind sterols perhaps at the second hydrophobic region. The second hydrophobic region has been identified by Yamamoto [22] as being important for sigma-1 receptor binding to (+)-pentazocine. It remains to be seen if the same region is also responsible for the binding of neurosteroids to sigma-1 receptors. So far, the sigma-2 receptor has not been cloned and its sequence remains unknown.

This review will briefly summarize the current understanding and major breakthroughs in the studies of sigma-1 receptors. Therefore, this review will not be extensive and not all references can be cited. The readers can check all the past reviews on this subject [8-10,23]. A most updated thorough description of most of the works mentioned in the present review will appear in a book [24]. The readers are encouraged to read the book, especially on the subjects that are not the authors' expertise such as in the area of cardiac function, immunity, and gastrointestinal function.

## PHYSIOLOGICAL AND PHARMACOLOGICAL ACTIONS

Although sigma/opioid receptors are purported by Martin *et al.*. [1] to mediate the psychotomimetic actions of certain opioids, the sigma receptors are not the sigma/opioid receptors (see HISTORY AND INTRODUCTION). Thus, the relationship of sigma receptors, in particular sigma-1 receptors, to psychotomimesis is unclear. In humans, high doses of (+)-pentazocine, a sigma-1 receptor ligand, have been reported by Keats and Telford [25] to cause a disturbed psychological state. There is no definitive clinical study that can provide direct link between sigma receptors, or sigma-1 receptors, to psychotomimesis. Although haloperidol is a sigma-1 receptor ligand, the affinity of haloperidol at dopamine D2 receptors is about the same as that at sigma-1 receptors [2,3,5,6]. The antipsychotic action of haloperidol is generally attributed to its activity at dopamine D2 receptors. Whether sigma receptors, or for that matter sigma-1 receptors, are involved in psychosis or other psychiatric disorders will be examined in a separate review [26].

In the age of proteomics, it is not unusual to search for the physiological role of a protein once the protein is identified regardless of whether it is identified from the purification, cloning, or structural co-existence with other proteins. The sigma-1 receptor was identified first with binding assays, albeit being originally thought to be sigma/opioid receptors, and was later identified by cloning. Thus researchers in the area of sigma-1 receptors have been performing a "reverse pharmacology" examining the biochemical and functional roles of a dextrorotatory benzomorphan- and neurosteroid-binding protein. Because the sigma-1 receptor is not a well-understood entity in terms of its signaling pathway, transduction mechanism, and structural interaction with other well-known proteins or receptors, the investigations in sigma-1 receptors have been typically exploratory and are largely ignored by the scientific communities that are used to investigating well-defined areas of research. The unknown identity of endogenous ligands for sigma-1 receptors also slows the progress of the investigation. Although sex hormones such as progesterone and testosterone have nM to submicromolar affinity at sigma-1 receptors [7,15,21], their roles as endogenous sigma-1 receptor ligands await further investigation and need to be fully established.

Despite the aforementioned drawbacks, some progresses on the physiological and pharmacological roles of sigma-1 receptors have been made in the past and even more so very recently.

The most clear-cut demonstration that sigma-1 receptors have a physiological role came from an electrophysiological study. Prior to this study, sigma-1 receptors merely remained a binding protein. Monnet and Debonnel [27,28] showed that in anesthetized rats, the neuronal firing in the CA3 region of hippocampus induced by NMDA was potentiated by sigma-1 receptor ligands like (+)-pentazocine. This effect of sigma-1 ligands is specific on the neuronal firings induced only by NMDA and not by kainate. Further, they showed that the (+)-pentazocine effect could be blocked by putative sigma-1 receptor antagonists including haloperidol. These series of studies by Monnet *et al.*. [e.g. 27,28] not only demonstrated a clear-cut agonist-antagonist action of ligands at physiologically relevant concentrations at sigma-1 receptors but also pointed out a very important aspect of the action of sigma-1 receptor agonist which still holds valid even till now: (+)-pentazocine has no detectable effect on its own but can potentiate the action of glutamatergic stimulation caused by NMDA. These results indicated that the biochemical action of sigma-1 receptors is modulatory in nature and that the consequence of the action of sigma-1 receptors may only be manifested when another biological system is first activated. Therefore, the study of sigma-1 receptors, although bearing an agonist-antagonist characteristic, may not be approached from the traditional concept of classical receptor theories. It has to be mentioned that at present, the molecular basis of this pioneering observation made by Monnet *et al.*. [27,28] on the potentiation of the NMDA action by sigma ligands has not been totally clarified and remains one of the most interesting questions in the area of sigma-1 receptor research.

Because of the successful demonstration of the physiological role of sigma-1 receptors in "modulating" the action of NMDA, Maurice *et al.*. [23,29] set forth to search for a behavioral relevance of Monnet *et al.*.'s finding [27,28]. The rationale was that if sigma-1 agonists like (+)-pentazocine or neurosteroids like pregnenolone sulfate can potentiate the action of NMDA in the hippocampus, perhaps the sigma-1 receptor agonists can reverse the behavioral deficit caused by blocking the NMDA receptor. Indeed, (+)-pentazocine and PRE-084 (2-(4-morpholino)ethyl-1-phenylcyclohexane-1-carboxylate hydrochloride; a sigma-1 ligand developed by the authors' laboratory) reversed the MK-801-induced deficit in the animal models of amnesia such as Morris water maze, special recognition in a Y-maze, retention of memory in passive avoidance step-down test [29]. Sigma-1 antagonists such as haloperidol or BMY-14802 (alpha-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine) blocked these effects caused by sigma-1 agonists. Importantly, in agreement with the observation of Monnet *et al.*.[27,28], the mnemonic studies indicated that sigma-1 receptor agonists have no effect of their own when tested by themselves. These memory tests conducted by Maurice *et al.*. [29] constitute the first evidence that sigma-1 receptors play certain physiological roles in animal behavior.

A surprise came later when Maurice and colleagues further examined the involvement of sigma-1 receptors in other animal models of mnemonic impairment. These included impairment elicited either by chemically treating the animals respectively with the nicotinic acetycholine receptor blocker mecamylamine [29], the calcium channel blocker nimodipine [30], and the $\beta$-amyloid$_{25-35}$ aggregate [31], or the model of genetically produced senescence-accelerated mice [32]. The surprise was that the sigma-1 receptor agonists are active in improving the mnemonic deficit elicited by all the above treatment procedures. Further, the sigma-1 receptor antagonists blocked the memory-improving action of sigma-1 agonists in all tests. Progesterone turned out to be a sigma-1 receptor antagonist in these behavioral tests, antagonizing the memory-improving effect caused by (+)-pentazocine or pregnenolone sulfate [31]. Importantly, in genetically senescent mice, the sigma-1 receptor agonists alone were active at improving the mnemonic capacity of senescent mice, suggesting an altered biological state in the senescent mice that can manifest the biochemical consequence caused by the sigma-1 receptor activation. Further, these results suggest that sigma-1 receptors are intrinsic molecules in the mnemonic processes of animals. These results also suggest a potential site of action of sigma-1 receptors in the cellular system to be discussed later (see CELLULAR AND BIOCHEMICAL STUDIES).

Initiating their projects totally from a different angle, Pasternak's group examined a possible role of sigma-1 receptors in pain modulation. Haloperidol has been known to potentiate the opioid-induced analgesia for many years and no satisfactory explanation is available. Although haloperidol is a dopamine D2 antagonist, it is also a sigma-1 receptor antagonist. The series of studies by Pasternak and co-workers [33-36] indicated that mu opioid receptor- and kappa opioid receptor-activated analgesia are potentiated by the sigma-1 receptor antagonist haloperidol and attenuated by sigma-1 receptor agonists such as (+)-pentazocine. Importantly, an antisense oligodeoxynucleotide designed against sigma-1 receptors eliminated these effects caused by the sigma-1 agonist or antagonist [34,36]. An interesting hypothesis was thus made by Pasternak and co-workers: "Sigma-1 receptors represent a tonic inhibitory tone on the mu and kappa receptor-mediated signaling pathways". The inhibition of the sigma-1 receptor tone by sigma-1 antagonists like haloperidol may thus increase the opioid-induced analgesia, and the enhancement of the sigma-1 receptor tone by sigma-1 agonist like (+)-pentazocine can attenuate opioid-induced analgesia. These suggest that other sigma-1 receptor antagonists without dystonic side effects like that caused by haloperidol may be useful adjunct agents to opioids in the treatment of pain. The reduction in the dose of opioids, if sigma-1 antagonist is used as a combination treatment, may reduce the development of opioid-induced undesirable side effects such as addiction. Dextromethorphan, an antitussive agent, is a sigma-1 receptor ligand and has been shown to potentiate opioid analgesia [37] just like a sigma-1 receptor antagonist did in Pasternak's studies [33-36]. However, high dose of dextromethorphan has been reported to cause psychosis [38,39] perhaps by being metabolized into dextrophan which can block the NMDA receptor channels just like phencyclidine. Perhaps developing dextromethorphan analogs without NMDA channel affinity would lead to potential sigma-1 antagonists that are safe and may potentiate opioid alangesia. The mechanism(s) underlying this interesting action of sigma-1 receptors in the modulation of pain perception is still under investigation.

Cocaine is a psychostimulant whose action involves a transport blockade of dopamine back to the synaptic terminals. A study by Kuhar and colleagues found that cocaine could bind to sigma-1 receptors with an affinity at about 2 μM which is close to the blood concentration of cocaine in cocaine addicts [40]. This finding raised a possibility that the actions of cocaine, at least partly, might be mediated by sigma-1 receptors. However, sigma-1 receptors are endoplasmic reticulum proteins and the affinity of cocaine, at least in its salt form, at sigma-1 receptors is in the μM range. It remains unknown at present whether cocaine can pass the plasma membrane in sufficient concentration to act on the sigma-1 receptor. Cocaine base can certainly pass the cell membrane. Cocaine hydrochloride has also been shown to cross the plasma membrane [41]. Further, a recent report indicates that sigma-1 receptor ligands can be transported inside the cells in an energy-dependent manner [42].

Inspired by the findings of Kuhar and colleagues [40], a few groups began to examine the possibility that certain actions of cocaine may be related to sigma-1 receptors. Ujike *et al.*. [43,44] demonstrated that behavioral sensitization caused by cocaine, which has been purported to relate to the reinforcing and addictive liability property of cocaine, was cross-sensitized to (+)-3-PPP, a sigma-1 receptor ligand. Further, the putative sigma-1 receptor antagonist BMY-14802 blocked the behavioral sensitization caused by cocaine. Ujike's group also demonstrated that the behavioral sensitization caused by another psychostimulant, methamphetamine, was antagonized by sigma-1 receptor antagonists [45]. It appeared that the sigma-1 receptors affect the development or acquisition of behavioral sensitization without affecting the expression of sensitization [44]. Using a more selective sigma-1 antagonist, MS-377 ((R-(+)-1-(4-chlorophenyl)-3-[4-(2-methoxyethyl)piperazin-1-yl]methyl-2-pyrrolidinone L-tartrate), another research group was able to replicate Ujike's finding [46]. Methamphetamine-induced behavioral sensitization in rats was blocked by MS-377 [46]. These results suggest that sigma-1 receptors play an important role in the acquisition of behavioral sensitization to psychostimulants in animals. However, more stringent protocols should be followed to replicate or confirm these findings, such as using individually housed animals and testing them in their home cages versus the test cage. Nevertheless, these results suggest the involvement of sigma-1 receptors in certain aspects of the action of cocaine or methamphetamine. The affinity of methamphetamine at sigma-1 receptors has not been reported in the literature. The affinity of cocaine at sigma-1 receptors, as mentioned before, is about 2 μM. It remains to be seen if the above observations on the behavioral sensitization to psychostimulants are the downstream consequence of direct binding of cocaine or methamphetamine to sigma-1 receptors or are the indirect consequence of these psychostimulants causing an increase of the synaptic concentration of dopamine, or the consequence of a combination of both.

Matsumoto's group examined the acute effect of cocaine and found that sigma-1 receptor antagonists blocked the lethality caused by cocaine [47,48]. They also found that the locomotor stimulation caused by acute cocaine might also be attenuated by sigma-1 receptor antagonists [49]. These effects are mediated via sigma-1 receptors because an antisense oligodeoxynucleotide directed against sigma-1 receptors blocked the protective effects exerted by the sigma-1 receptor antagonists [50]. More studies are underway to examine the exact mechanism(s) via which sigma-1 antagonists counteract the locomotor stimulation or toxicity induced by acute cocaine administration.

To further provide evidences that sigma-1 receptors are involved in the action of cocaine, Maurice and colleagues [51] examined the role of sigma-1 receptors in the conditioned place preference elicited by cocaine. Interestingly, like previous studies showing that sigma-1 agonists typically caused no observable behavioral effect when administered by themselves, Maurice's results with the conditioned place preference also showed the same phenomenon: sigma-1 agonist treatment alone did not cause a conditioned place preference. However, the conditioned place preference caused by cocaine or another dopamine transporter inhibitor BTCP (N-[1-(2-benzo(b)thiophenyl) cyclohexyl]piperidine), which has no sigma receptor affinity, was blocked by the sigma-1 receptor antagonist [52]. These results render further support to the notion that sigma-1 receptors are involved in the action of cocaine, specifically in this case the acquisition of the conditioned place preference. Given that sigma-1 receptors are apparently involved in the behavioral sensitization caused by cocaine and methamphetamine and that sigma-1 receptors are also involved in the acute action of cocaine, it is reasonable to speculate that sigma-1 receptors may be involved in the self-administration of cocaine or methamphetamine. So far, no such a report is available in the literature.

A recent development in exploring the behavioral role of sigma-1 receptors is in that sigma-1 receptor agonists apparently act as antidepressants in animal models of depression including forced swimming [53-55]. The rationale for testing sigma-1 receptor agonists as antidepressants has a bearing with the fact that almost all antidepressants, including tricyclic compounds like imipramine, SSRI's (Selective Serotonin Reuptake Inhibitor) and the MAO (monoamine oxidase) inhibitor deprenyl, possess high to moderate affinity at sigma-1 receptors [2,56-58]. This may represent a fruitful area that deserves systematic examinations. If it turns out in the future that the sigma-1 receptors are the underlying molecular substrate that mediates the action of antidepressants, the basic mechanism of action of sigma-1 receptors may provide a new avenue for developing more effective antidepressants.

A recent report indicated that the spreading depression observed within 3-5 min after cerebral ischemia was blocked by sigma receptor ligands and the blockade was attenuated by sigma-1 receptor antagonists [59]. Whether the effect involves sigma-1 or sigma-2 receptor is unknown at present. The exact mechanism underlying this interesting and important action of sigma ligands deserve further investigation, as the ligands may be of therapeutic importance in the treatment against migraine, stroke, or head trauma [59].

## CELLULAR AND BIOCHEMICAL STUDIES

A critical question concerning this burgeoning area of research on sigma-1 receptors is: What exactly is the sigma-1

receptor doing that it may be involved in the mnemonic processes, pain perception, and behavioral sensitization? Many studies have been undertaken to answer this question. The task has not been easy because the sequence of sigma-1 receptor does not resemble that of any mammalian protein. Therefore no precedent example can be followed that may provide a potential clue. Most of the studies are thus exploratory in nature based on the limited information obtained from animal behavioral studies or whole animal electrophysiological studies mentioned above. Nevertheless, interesting results have been obtained.

Because sigma receptors are involved in the action of NMDA and because NMDA receptors are involved in the regulation of dopamine release, Monnet's [60] and mostly Werling's group [61-65] have examined systematically the role of sigma-1 receptors in the regulation of release of classical neurotransmitters including the dopamine, acetylcholine and norepinephrine in brain slices. Using microdialysis techniques, Matsuno's group also reported an increased release of acetycholine in the frontal cortex of rat brain after administration of sigma-1 receptor agonists [66]. Horan *et al.*. confirmed this in a recent study [67]. Notably, the concentrations of sigma-1 agonists used in these reports were typically in the nM range and an agonist-antagonist relation clearly established in the study. These studies together constitute the first report of a clear-cut agonist-antagonist relationship in the action of sigma-1 receptors using an *in vitro* preparation. The exact mechanism(s) underlying the action of sigma-1 receptors in regulating the neurotransmitter release awaits further investigation. Considering that sigma-1 receptors are endoplasmic reticulum proteins, the involvement of sigma-1 receptors in regulating the depolarization- or ligand-induced neurotransmitter release must involve the communication between the plasma membrane and the endoplasmic reticulum. Therefore the intracellular signal transmitting from the plasma membrane leading to the activation of sigma-1 receptors at the endoplasmic reticulum will be of interest to many researchers. Morin-Surun *et al.*. [68] proposed that protein kinase C might play an important role in the activation of sigma-1 receptors. They found that protein kinase C may cause the phosphorylation of sigma-1 receptors and that phosphorylated sigma-1 receptors can apparently translocate from inside the cell to the plasma membrane [68]. Werling's group [69] further confirmed the involvement of protein kinase C in the activation of sigma-1 receptors in eliciting neurotransmitter release by employing selective protein kinase C inhibitors in their studies. Werling's group recently found that protein kinase Cβ specifically involves in the activation of sigma-1 receptors [69].

The information, as shown above, calls for an urgent need to identify the basic molecular action that sigma-1 receptors may cause, specifically within the context that sigma-1 receptor agonists do not cause an apparent effect unless a co-factor or a system is activated or first present in active form. Several recent discoveries have provided a critical link to address this issue.

Because Maurice's behavioral studies indicated that sigma-1 receptor activation can reverse the behavioral deficit induced by the receptor/channel blockers MK-801, mecamylamine, and nimodipine, and because all three blockers can reduce the intracellular calcium level, Hayashi *et al.*. [70] speculated that sigma-1 receptors may play a role in the regulation of intracellular calcium concentration, thus able to counteract the action of these three calcium channel blockers. Because sigma-1 receptors are not co-localized with any of the receptors/channels that the blockers act upon, the speculation requires that sigma-1 receptors work inside the cell to counteract the actions of all three different types of blockers. This is a reasonable speculation because sigma-1 receptors have been shown to reside mainly at the microsomal fractions in earlier studies [e.g., 71].

Previous studies from Brent *et al.*. [72] and Eilam and co-workers [73,74] have suggested that sigma receptors may be related to protein kinase C and IP3 (Inositol (1,4,5)-trisphosphate) respectively in the brain synaptosome preparation and cardiomyocytes. Using a mammalian neuron-like cell line NG-108, Hayashi *et al.*. [70] found that sigma-1 receptor agonists like (+)pentazocine, PRE-084, and pregnenolone sulfate, while by themselves exhibiting no effect at all in NG-018 cells, can however potentiate the bradykinin-induced increase in intracellular calcium concentration [70]. Sigma-1 receptor antagonists blocked the potentiation induced by sigma-1 receptor agonists. Further, antisense oligodeoxynucleotide directed against the cloned sigma-1 receptor attenuated the potentiation [70]. They also demonstrated that loci of this action of sigma-1 receptors are at the endoplasmic reticulum because thapsigargin, which depletes the endoplasmic reticulum calcium, can block the action caused by sigma-1 agonists. This study clearly suggested that the action of sigma-1 receptors is related to intracellular calcium signaling at the endoplasmic reticulum. The fact that sigma-1 agonists did not cause an increase of IP3 formation supports this notion [70]. Further, an agonist-antagonist relationship was observed in the regulation of this signaling pathway and the agonists have no apparent effect on their own. In the study, they also found that sigma-1 receptor agonists cause the translocation of sigma-1 receptors from microsomes to the plasma membrane and nucleus. This observation is in agreement with Morin-Surun *et al.*. [68] showing that upon the activation of protein kinase C, sigma-1 receptors can be phosphorylated and translocated from inside the cell to the plasma membrane.

Hayashi and Su [75] further evaluated the molecular mechanism governing the regulation of intracellular calcium signaling at the endoplasmic reticulum mediated by sigma-1 receptors in NG-108 cells. They found that sigma-1 receptors form a trimeric complex with other two proteins on the endoplasmic reticulum: the IP3 receptor and the ankyrin isomer 220. Ankyrins are cytoskeletal adaptor proteins linking spectrin to F-actin and have been shown to play important roles in the organization of ion channels and proteins at the plasma membrane and at the axonal nodes of Ranvier in neurons [76]. Upon the stimulation elicited by sigma-1 receptor agonists like (+)-pentazocine and cocaine, sigma-1 receptor/ankyrin dissociates as a dimer from IP3 receptors, which remain on the endoplasmic reticulum, and translocates to the plasma membrane and nucleus. This action of (+)-pentazocine and cocaine were blocked by the sigma-1 receptor antagonist, which appeared to do so indirectly by causing sigma-1 receptors to dissociate from ankyrin which remains coupled to IP3 receptors on the

endoplasmic reticulum [75]. Sigma-1 receptor agonists caused no detectable effect when tested by themselves in the regulation of calcium signaling at the endoplasmic reticulum [70]. Apparently, all the molecular events involving sigma-1/ankyrin translocation as mentioned above took place in Hayashi *et al.*.'s calcium experiment [70] without causing detectable effects in the intracellular calcium signaling when the NG-108 cells were at their resting state. As mentioned before in a speculation, a system must be activated first to unveil or cause a manifestation of the physiological significance of that caused by the sigma-1 receptor agonists; in this case, being the translocation of the sigma-1 receptor/ankyrin dimeric complex from IP3 receptors at the endoplasmic reticulum. Indeed, in the resting state when NG-108 cells received no stimulus, the dissociation of sigma-1 receptor/ankyrin complex caused by sigma-1 receptor agonists produced no detectable influence on the efflux of calcium from endoplasmic reticulum [75]. However, when the cells were stimulated by bradykinin, the dissociation of sigma-1 receptor/ankyrin caused by sigma-1 receptor agonists makes a difference. The dissociation of the sigma-1 receptor/ankyrin complex from IP3 receptors on the endoplasmic reticulum causes an increase in the binding of IP3, thus enhancing the calcium efflux from these receptors [75]. Thus, apparently, sigma-1 receptors and associated ligands help create a "supersensitized state" to facilitate the amplication of IP3 signaling at the endoplasmic reticulum. This amplification can only be manifested when IP3 is present in sufficient concentrations, such as that caused by bradykinin stimulation of the cell.

The above results, when taken together with the observation that sigma-1 receptors translocate to the plasma membrane and nucleus, led us [77] to propose a hypothesis that sigma-1 agonists may mediate certain actions of cocaine by participating in the structural reorganization of critical cellular substrates. Because ankyrins are enriched in the dendritic spines [78] and because cocaine increases the density of dendritic spines [79], we speculated that sigma-1/ankyrin may mediate the formation of dendrites induced by cocaine [77]. The cocaine-induced increase of dendritic spines has been proposed to relate to cocaine-induced craving and addiction [79]. Because a high density of sigma-1 receptors were also observed in growth cones of NG-108 cells [75], we speculated recently that sigma-1 receptors may be involved in neurite sprouting. Indeed, we found that sigma-1 receptors are intrinsic molecules playing a critical role in signaling cascade of the nerve growth factor and proposed that sigma-1 receptors may mediate the action of certain antidepressants by potentiating the neurite sprouting caused by the nerve growth factor [80]. Notably, sigma-1 receptor agonists alone in that study did not cause neurite sprouting unless the cells were activated by nerve growth factor [80].

Sigma receptors have been shown to modulate potassium channels. A major discovery was made recently by Jackson and colleagues showing that in *Xenopus* oocytes sigma-1 receptors regulate potassium channels (Kv1.4 or Kv1.5) on the cell membrane by forming a ligand-regulated potassium channel subunit [81]. The study also demonstrated that even without exogenously added sigma-1 receptor ligands, the apparent protein-protein interaction between sigma-1 receptors and the Kv1.4 channels was sufficient to cause the channel inactivation. As sigma-1 receptors modulate the potassium channel in a fashion similar to that caused by the β subunits of voltage-gated channels, Jackson and colleagues [81] suggested that sigma-1 receptors might be a family member of the β subunits. However, the β subunit acts like a pendulum blocking the channel cavity. Whether the topology of sigma-1 receptors might cause the same effect is unknown at present. In contrast to that seen in the NG-108 cells where most sigma-1 receptors are localized inside the cells or at plasmalemmal areas under the contacting plasma membrane [75], the majority of sigma-1 receptors in oocytes are localized on the plasma membrane and apparently contain two transmembrane regions [81]. It is not known at present whether the discrepancy may have arisen from the different developmental or pathophysiological state of the cells employed in the studies. A detailed examination on brain slices or primary neurons may provide a potential explanation.

## PERSPECTIVES AND CONCLUSION

Originally identified as a binding site in the brain, the sigma-1 receptor has now been recognized as a non-opioid, non-phencyclidine receptor which clearly plays certain important roles in biological systems. These roles include calcium signaling, neurotransmitter release, ion channel regulation, cognition, pain, depression, movement disorder, cardiac, immune, and gastrointestinal functions, and perhaps neuroplasticity and addiction. Advances in understanding the molecular mechanism of sigma-1 receptors in the past few years have paved an avenue or clue to explore a possibility that sigma-1 receptors may play a very basic biological role in the living system. That basic role played by sigma-1 receptors may affect many facets of the living system. Because sigma-1 receptors translocate in cells [82], we believe that the basic biological role of sigma-1 receptors is deeply related to this translocation [83] and that the realization of the basic role played by sigma-1 receptors may have to be pursued in this perspective. Lastly, results from biochemical and behavioral studies suggest a hypothesis that sigma-1 receptors act as intracellular amplifiers for signal transductions involving the dompaminergic, glutamatergic, IP3-related metabotropic, or nerve growth factor-related systems. Whether the translocation of sigma-1 receptors is related to their potential roles as intracellular amplifiers remains to be investigated.

## ABBREVIATIONS

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| BMY-14802 | = | Alpha-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperizine |
| BTCP | = | N-[1-(2-benzo(b)thiophenyl)cyclohexyl] piperazine |
| DTG | = | 1,3-Di(2-tolyl)guanidine |
| IP3 | = | Inositol (1,4,5)-trisphosphate |
| MAO | = | Monoamine oxidase |
| MS-377 | = | ((R-(+)-1-(4-chlorophenyl)-3-[4-(2-methoxyethyl)piperazin-1-yl]methyl-2-pyrrolidinone L-tartrate |

| | | |
|---|---|---|
| NMDA | = | N-Methyl-D-aspartate |
| 3-PPP | = | 3-Hydroxyphenyl-N-(1-propyl)-piperidine |
| PRE-084 | = | 2-(4-morpholino)ethyl-1-phenylcyclo-hexane-1-carboxylate hydrochloride |
| SFK-10047 | = | N-Allyl-normetazocine |
| SSRI | = | Selective Serotonin Reuptake Inhibitor |

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