

σ -1 Receptor Ligands

Potential in the Treatment of Neuropsychiatric Disorders

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Abstract

The σ receptor was originally proposed to be a subtype of the opioid receptor. However, it is now clear that σ receptors are unique non-opioid, non-phencyclidine brain proteins. Two types of σ receptor exist, the σ -1 receptor and the σ -2 receptor. σ -1 receptors have been cloned and their distribution, physiological functions and roles in signal transduction were recently characterised. Certain sex hormones in the brain (neurosteroids) are known to interact with σ -1 receptors. σ -1 receptors regulate glutamate NMDA receptor function and the release of neurotransmitters such as dopamine. They are thus proposed to be involved in learning and memory as well as in certain neuropsychiatric disorders.

Selective σ -1 receptor ligands have been suggested to represent a new class of therapeutic agents for neuropsychiatric disorders, although none have yet been introduced into therapeutic use. Early studies showed that psychotomimetic benzomorphans, as well as several antipsychotics, can bind to σ -1 receptors. As a result of these findings, σ -1 receptor ligands have been proposed as being of potential use in the treatment of schizophrenia. Nevertheless, the relationship of σ -1 receptors to the underlying pathogenesis of schizophrenia is still unclear. σ -1 receptor ligands have failed to improve acute psychotic symptoms of schizophrenia in clinical trials, but, interestingly, a few studies have shown an improvement in negative symptoms in schizophrenic patients.

A number of preclinical studies have shown that selective agonists of σ -1 receptors affect higher-ordered brain functions such as learning and memory, cognition and mood. These studies indicate that σ -1 receptor agonists may exert therapeutic effects in depression and senile dementia. Indeed, the σ -1 receptor agonist igmesine, has been shown to improve depression in a clinical trial. The most distinctive feature of the action of σ -1 receptor ligands is their 'modulatory' role. In behavioural studies of depression and memory, they exert beneficial effects only when brain functions are perturbed.

Given the recently accumulated preclinical and clinical data, it is time to reconstruct the concept of σ -1 receptors and the associated pathophysiological conditions that ligands of these receptors target. This would allow clinical trials to be performed more efficiently, and the results may confirm a long-specified possibility that σ -1 receptor ligands represent a new class of therapeutic agents for neuropsychiatric disorders.

σ receptors were originally proposed by Martin et al.^[1] to be a subtype of opioid receptor. Since benzomorphans such as SKF-10047 (N-allylnormetazocine) and pentazocine are known to bind

selectively to σ receptors and cause delusions and hallucinations in animals and humans,^[1-6] Martin et al.^[1] speculated that these drugs have a psychotomimetic action through an interaction with σ receptors. However, a series of later experiments demonstrated that the σ receptor is insensitive to naloxone, a universal antagonist of opioid receptors,^[7,8] and confirmed that the σ receptor is a non-opioid receptor. However, because SKF-10047 can bind to the phencyclidine (PCP) site on glutamate NMDA receptors and, conversely, PCP can bind to the σ receptor, confusion arose between the σ receptor and the PCP site on NMDA receptors.^[9] Further experiments using more selective ligands for the σ receptor, such as (+)-3-(3-hydroxyphenyl)-N-propylpiperidine [(+)-3-PPP] and for the PCP site, such as thienylcyclohexylpiperidine (TCP), showed that the binding sites and brain distribution of σ and NMDA receptors are different,^[10-13] leading to the conclusion that the σ receptor is a non-opioid, non-PCP unique brain-enriched receptor.

Data from receptor binding studies indicate that at least two subtypes of σ receptor exist: σ -1 (high affinity site) and σ -2 (low affinity site) receptors.^[14] The σ -1 receptor was cloned in 1996.^[15] The cloned protein contains an endoplasmic reticulum (ER) retention signal (therefore residing at the ER) and at least one putative transmembrane domain.^[15-18] The σ -2 receptor has not been cloned.

In this article, we focus on the σ -1 receptor. As mentioned above, some benzomorphanes that can bind to the σ -1 receptor are known to cause psychosis in humans.^[2,4-6] Furthermore, it was discovered in the 1980s that some antipsychotics, especially haloperidol, have relatively high affinities for σ -1 receptors.^[7] Thus, σ -1 ligands were originally proposed to be a new class of antipsychotics for the treatment for schizophrenia. However, until the early 1990s, methods for studying the σ -1 receptor were confined to mainly receptor binding assays and there were no established biochemical and behavioural tools to characterise the agonist-antagonist action of σ -1 ligands and their implications for certain neurophysiological conditions. In addition, endogenous ligands, intracellular signal transduction and receptor localisation had not been clarified. These impeded the identification of the specific therapeutic targets of σ -1 receptor ligands and their

introduction into clinical trials. However, recently accumulated evidence on σ -1 receptors from basic and preclinical research has resulted in a significant advance in our understanding of this orphan receptor. In particular, behavioural studies using animals have contributed greatly to understanding the physiological function of σ -1 receptors.

In this article, findings on σ -1 receptors from preclinical and clinical research are summarised, and the physiological function of σ -1 receptors, as well as the potential therapeutic targets of σ -1 receptor ligands in clinical pharmacotherapy, discussed.

1. σ -1 Receptors

1.1 Pharmacology

The σ -1 receptor was originally identified using a dextrorotatory isomer of the benzomorphan SKF-10047 [(+)-SKF-10047],^[7,9-11,13] and further characterised by more selective compounds such as (+)pentazocine.^[19] (-)-Isomers [e.g. (-)-SKF-10047] have significantly lower affinities for the σ -1 receptor.^[9,14]

It is now known that many structurally diverse compounds can bind to the σ -1 receptor (table I). Interestingly, several clinically used psychotropic drugs such as haloperidol, imipramine and SSRIs can bind to the receptor with low nanomolar affinity.^[7,20-23] Some σ -1 receptor ligands such as haloperidol, NE-100 and MS-377 have been shown to block, especially in *in vivo* studies, the action of (+)pentazocine and other selective σ -1 ligands and, therefore, have been categorised as σ -1 receptor antagonists.^[24,25] However, it is still unclear exactly which pharmacological actions exerted by these therapeutic compounds are mediated by the σ -1 receptor.^[7,20,22,23] As such, in drug development, in addition to receptor binding assays, a thorough evaluation of σ -1 ligands by recently established *in vitro* and *in vivo* assays (see sections 1.3 and 2.1) is necessary, as evidence from binding assays alone may not be sufficient to fully characterise the ligand. In other words, more criteria should be applied when interpreting a pharmacological action of 'so-called' σ receptor ligands, as the compounds that have affinity for the σ binding site may not always be selective and functional.

Table I. σ-1 receptor ligands

Compound	Affinity ^a	Subtype selectivity	Function	Comment
Benzomorphans				
(+)SKF-10047	+++	σ-1	Agonist	Also binds to the PCP site
(+)Pentazocine	+++	σ-1	Agonist	Highly selective agonist
(+)-3-PPP	+++	σ-1	Agonist	Dopamine receptor agonist
Antipsychotics				
Haloperidol	+++	σ-1/2	Antagonist	Dopamine D ₂ receptor antagonist
Chlorpromazine	++	?	?	D ₂ antagonist
Nemonapride	+++	σ-1/2?	?	D ₂ antagonist
Antidepressants				
Imipramine	++	σ-1	Agonist	Monoamine reuptake inhibitor
Fluoxetine	++	σ-1	Agonist	SSRI
Fluvoxamine	+++	σ-1	Agonist	SSRI
Neurosteroids				
Progesterone	+++/**	σ-1	Antagonist	Partial agonist in some assays
Testosterone	+++/**	σ-1	?	
Pregnenolone sulphate	+	σ-1	Agonist	
PB-008 (dihydroepiandrosterone sulphate)	+	σ-1	Agonist	
Cocaine	+	σ-1	Agonist	Dopamine transporter inhibitor
Other synthetic compounds				
DTG	+++	σ-1/2	Agonist?	
BD-1008	+++	σ-1/2	Antagonist	
PRE-084	+++	σ-1	Agonist	
NE-100	+++	σ-1	Antagonist	
Dup-734	+++	σ-1	Antagonist?	
Igmesine (JO-1784)	+++	σ-1	Agonist	Improves depression ^{[26]b,d}
SA-4503	+++	σ-1	Agonist	
SR-31747A	+++	?	?	High affinities for both σ-1 receptor and C8-C7 sterol isomerase
MS-377	+++	σ-1	Antagonist	
OPC-14523	+++	σ-1/2	Agonist?	Serotonin 5-HT _{1A} receptor agonist
Rimcazole (BW-234U)	+	σ-1?	Antagonist	Ineffective against the positive symptoms of schizophrenia ^{[27]b}
BMS-181100 (BMY-14802)	++	σ-1	Antagonist	Ineffective against the positive symptoms of schizophrenia ^{[28]c}
Panamesin (EMD-57445)	+++	σ-1?	Antagonist?	Effective against the positive/negative symptoms of schizophrenia ^{[29,30]d} Metabolite is a dopamine antagonist
Eliprodil (SL-82.0715)	+++	?	?	Effective against negative symptoms of schizophrenia ^{[31]d} Glutamate NMDA polyamine site antagonist

a Inhibition constant (K_i) values, where +++ indicates <50 nmol/L, ++ indicates <500 nmo/L and + indicates <10 μmol/L.

b Double-blind study.

c Single-blind study.

d Open-label study.

3-PPP = 3-(3-hydroxyphenyl)-N-propylpiperidine; **DTG** = 1,3-di-o-tolylguanidine; **PCP** = phencyclidine.

Sex steroid hormones such as progesterone and pregnenolone sulphate are known to bind to the σ -1 receptor.^[32] These hormones are synthesised *de novo* in the brain (and are termed neurosteroids) and are known to regulate NMDA and GABA_A receptor-coupled channels.^[33] Specifically, progesterone possesses a significant affinity for the σ -1 receptor at physiologically relevant concentrations; the affinity of progesterone at the σ -1 receptor has been shown to be about 250 nmol/L in binding studies.^[32,34] Brain levels of neurosteroids are known to be affected by aging, emotion and certain pathophysiological states such as dementia and depression.^[33,35-37] Several behavioural and physiological experiments suggest the involvement of σ -1 receptors in certain behaviours caused by neurosteroids.^[38-41] The physiological relevance of neurosteroid binding to σ -1 receptors has been under intense investigation.

1.2 Distribution

σ -1 receptors are present in high density in the CNS. The early experiments using binding assays and autoradiography suggested that σ -1 receptors are enriched in several brain regions including the cerebellum, and exist also at a high density in the liver and adrenal gland.^[10-12,42-44]

σ -1 receptors are enriched in microsomal membranes from the brain,^[45] suggesting that they may localise on the endoplasmic reticulum (ER).

After the cloning of the σ -1 receptor, it became possible to measure the mRNA and protein levels of σ -1 receptors using nucleotide probes or antibodies.^[46,47] mRNA of σ -1 receptors is expressed at moderate levels in the brain and several peripheral organs including the stomach, liver, adrenal gland and testis.^[15-17] However, protein levels measured by a specific antibody against σ -1 receptors exhibit quite a different pattern (figure 1),^[48,49] with the level being highest in the brain, spinal cord and peripheral nerves, but is lower in peripheral organs including the liver and adrenal gland. Furthermore, in the brain, the σ -1 receptor protein level is high in the frontal cortex, hippocampus, and striatum, but lower in the cerebellum.^[48,49] Therefore, the distribution of the σ -1 receptor protein is much more restricted than previously reported. The distribution of σ -1 binding sites as originally characterised by

tritiated benzomorphans may contain multiple sites including the σ -1 receptor.^[50-52]

1.3 Effect on Cellular Function

As mentioned in section 1.2, recent studies using a specific antibody against the σ -1 receptor suggest that the receptor exists predominantly on the ER, especially the smooth ER.^[47,53] In the rat primary neuron, we found that the majority of σ -1 receptors

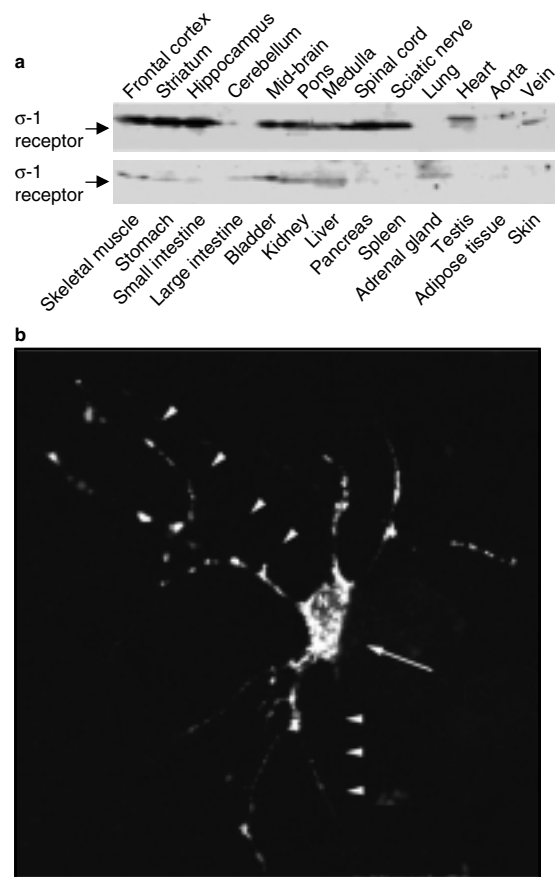


Fig. 1. Cellular and whole body distribution of σ -1 receptors. **(a)** Body distribution of σ -1 receptors in the mouse. Protein extracts from each organ or region were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and blotted by a σ -1 receptor antibody. **(b)** A rat brain primary neuron expressing yellow fluorescent protein (YFP)-tagged σ -1 receptors (shown in white). EYFP-N vectors® (clontech) containing σ -1 receptor cDNA were transfected to neurons from the postnatal rat brain. The cell body is indicated by the arrow and the dendrites by the arrowheads. N = nucleus.

are localised on the ER of the cell body and the dendrites, and are highly clustered (figure 1).^[48] These results are consistent with the enrichment of σ -1 binding sites in brain microsomes^[45] and with the existence of an ER retention motif on the N-terminus of the cloned σ -1 receptor.^[15]

Recent studies using green fluorescence protein-fused σ -1 receptors demonstrated that the receptors localise on lipid droplets associated with the ER in addition to being on the ER reticular network.^[1,54,55] Functional dominant negative σ -1 receptors failed to target ER-lipid droplets, which led to retention of neutral lipids and cholesterol in the ER, a decrease of cholesterol in plasma membranes and a bulbous aggregation of ER.^[54] The ER synthesises most lipids inside cells and exports them to the periphery of cells for the constitution of the plasma membrane. Therefore, these findings suggest that σ -1 receptors on the ER regulate the compartmentalisation and export of ER lipids, and the resultant are lipid compositions in the plasma membranes. Interestingly, upon stimulation with σ -1 receptor agonists, σ -1 receptors can translocate at the ER (i.e. from ER lipid droplets to periphery ER networks that are close to the plasma membrane or to the tips of neurites^[55]). The physiological role of σ -1 receptor translocation is unknown at present; however, because σ -1 receptors specifically target ER lipid droplets and compartmentalise neutral lipids, the dynamic translocation of the receptors might affect lipid transport and distribution in neuronal cells.

Localisation of σ -1 receptors on the ER is in perfect agreement with σ -1 receptors regulating Ca^{2+} signaling *via* inositol 1,4,5-trisphosphate (IP3) receptors on the ER.^[47] σ -1 receptor agonists were found to potentiate IP3-induced Ca^{2+} mobilisation in a biphasic dose-dependent manner in a neuronal cell line.^[47] The regulation is mediated by the association of the σ -1 receptor with cytoskeletal proteins on the ER.^[53]

In addition to its action at the ER, the σ -1 receptor can also modulate several physiological and cellular events on the plasma membrane (table II). The receptor regulates ion channels such as K^+ channels,^[56] NMDA receptors^[57-62] and voltage-dependent Ca^{2+} channels.^[47,63] It is suggested that the σ -1 receptor inhibits the K^+ channel current by a direct coupling as a modulatory subunit.^[56] Further re-

search is needed to understand the mechanisms by which the σ -1 receptor modulates diverse types of ion channels on the plasma membrane.

As mentioned in section 1.1, tricyclic antidepressants and SSRIs possess high affinity for the σ -1 receptor.^[7,20,22,23] We recently found that these antidepressants potentiate nerve growth factor-induced neurite growth in PC-12 cells *via* σ -1 receptors.^[64] Therefore, the affinity of these antidepressants for the σ -1 receptor may contribute to certain pharmacological actions, specifically their neurotropic action.^[64,92]

Several lines of research demonstrated that the σ -1 receptor modulates neuronal firing and neurotransmitter release.^[41,65-67,93-96] Monnet et al.^[66] found that σ -1 receptor ligands, although exhibiting no effect by themselves, selectively enhance NMDA-induced neuronal firing in the CA3 region of hippocampus in rats. σ -1 receptor ligands also modulate NMDA-mediated neuronal firing in the CA1 region in a biphasic dose-response manner.^[65] A 2-day treatment with σ -1 receptor agonists markedly increases neuronal firing of serotonergic neurons in the dorsal raphe nucleus.^[67] Interestingly, a selective σ -1 receptor agonist SA-4503 potentiates dopamine neuronal activity in ventral tegmental area, but suppresses it in substantia nigra pars compacta in the rat brain.^[68] On the other hand, σ -1 receptor ligands were shown to modulate depolarisation-induced dopamine release from brain slices.^[94] Nuwayhid and Werling^[95] recently demonstrated that σ -1 receptor agonists regulate NMDA-induced dopamine release from rat striatal slices *via* the protein kinase C pathway. σ -1 receptor agonists and neurosteroids, on the other hand, potentiate NMDA-induced noradrenaline release from rat hippocampus slices.^[41] It has also been shown, using *in vivo* microdialysis, that σ -1 receptor agonists increase spontaneous acetylcholine release in the frontal cortex.^[96]

Although the exact molecular action of the σ -1 receptor remain unclear, the receptor appears to regulate neuronal excitability by modulating plasma membrane potentials and/or intracellular Ca^{2+} signaling. It should be emphasised that most of the pharmacological effects of σ -1 receptor ligands are 'modulatory'. σ -1 receptor agonists did not cause Ca^{2+} mobilisation, neuronal firing and dopamine

release by themselves in the above-mentioned studies;^[47,66,95] they exerted their modulatory action when the IP3 receptor, K⁺ channel or NMDA channel was activated by transmitters or depolarisation^[47,56,66,95] (also see review by Su and Hayashi^[97] for details). Taken together, σ -1 receptors appear to play an important role in the CNS as a modulator or an amplifier of signal transduction in systems such as NMDA channel activity, Ca²⁺ signalling and NGF-induced cell differentiation.^[97] These systems have recently been shown to be essential factors in the formation of neural plasticity.^[98] Thus, we pro-

pose that σ -1 receptors might be involved in regulating the remodelling of neuronal membranes.^[54,55,99]

2. Effects of σ -1 Receptor Ligands in Animal Models of Behaviour

σ -1 receptor ligands have been tested in several animal models of behaviour. Actions of σ -1 receptor ligands include: (i) improvement of memory and cognitive function; (ii) antidepressant-like actions; and (iii) modulation of psychostimulant-induced behaviour.

2.1 Drug-Induced Amnesia

It is well established that cholinergic and glutamatergic (especially via the NMDA receptor subtype) neurotransmitter systems play a crucial role in the neurophysiological processes underlying learning and memory.^[100,101]

Selective σ -1 agonists have been shown to reverse amnesia induced by acetylcholine muscarinic and nicotinic receptor antagonists in animals.^[69,102] It is interesting that σ -1 receptor agonists including (+)pentazocine, (+)SKF-10047 and 1,3-di-o-tolylguanidine (DTG) exert an anti-amnesic action when administered either at pre-training, immediately after training or before retention test time points of mnemonic examinations.^[69,102,103] These results suggest that σ -1 receptor agonists can improve acetylcholine-related cognitive processes encompassing all phases of memory formation: acquisition, consolidation and retention.

Selective σ -1 agonists also improve amnesia induced by a NMDA receptor antagonist or by a Ca²⁺ channel antagonist.^[70,71] Using different behavioural tests, Maurice et al.^[70] demonstrated that σ -1 receptor agonists produce significant attenuation of short- and long-term memory disturbances induced by dizocilpine (MK-801). This action was blocked by NE-100, a σ -1 receptor antagonist.

Notably, in all behavioural tests for learning and memory, σ -1 receptor agonists again show a modulatory pharmacological action. They show anti-amnesic action only when memory and cognition are impaired by chemicals that inhibit neural transmission. By themselves, they do not further augment memory in normal animals. It is reported that σ -1 receptor ligands also show beneficial effect against

Table II. Modulatory pharmacological action of σ -1 receptor ligands

Parameter	System/model effected	Effect of σ -1 agonists
<i>In vitro</i> studies		
Ion channel activity ^[47,56-64]	Glutamate NMDA receptor	Potentiated/suppressed
	Ca ²⁺ channel	Suppressed
	IP3 receptor	Potentiated
	K ⁺ channel	Suppressed
Neurotransmitter release ^[39,63-65]	Dopamine	Suppressed
	Noradrenaline	Potentiated
Neuron firing ^[39,66-68]	Acetylcholine	Potentiated
	Glutamatergic (NMDA) neuron in hippocampus	Potentiated
	Serotonergic neuron in dorsal raphe nucleus	Potentiated
	Dopaminergic neuron in midbrain	Potentiated/suppressed
Behavioural studies		
Amnesia	Y-maze test and passive avoidance ^[39,40,49,67,69-72]	Improved
	Forced swimming test ^[49,73-77]	Improved
Depression and stress	Conditioned fear stress ^[49,78]	Improved
	Stress-induced colon contraction ^[1,79,80]	Improved
	PCP-induced cognitive dysfunction ^[24,25]	Inhibited by antagonists
Psychosis/drug-dependence	Psychostimulant-induced behavioural sensitisation ^[81-83]	Inhibited by antagonists
	Cocaine-induced conditioned place preference ^[84]	Inhibited by antagonists
	Cocaine toxicity ^[85-88]	Inhibited by antagonists
	Morphine-induced analgesia ^[89-91]	Inhibited

IP3 = inositol 1,4,5-triphosphate; **PCP** = phencyclidine.

memory impairment induced by physiological processes such as aging.^[72]

σ -1 ligands may not cause the adverse effect, such as peripheral cholinomimetic responses, that are often associated with acetylcholinesterase inhibitors (the current gold standard treatment for dementia).

Because σ -1 ligands improve amnesia induced by Ca^{2+} , acetylcholine or NMDA channel blockers, intracellular Ca^{2+} appears to be a candidate as a common factor regulated by σ -1 receptors. This possibility has been further investigated^[104] and it has been found that σ -1 receptors regulate Ca^{2+} release from intracellular Ca^{2+} storage sites.^[47,53] Furthermore, it has been shown that injections of inhibitors of intracellular Ca^{2+} release into the brain abolish the effects of σ -1 receptor agonists in behaviour tests.^[104]

Cognitive function depends partly on neurosteroid levels, which are decreased by aging. It has been shown that levels of pregnenolone sulphate are significantly lower in the hippocampus of aged (24-month) than in young (~3-month) male animals.^[105] Low levels of pregnenolone sulphate in the hippocampus are correlated with poor performance in spatial memory tasks.^[105] Central or systematic administration of PB-008 (dihydroepiandrosterone [DHEA] sulphate) and pregnenolone sulphate improves/enhances mnemonic performances.^[105] These actions of neurosteroids involve NMDA-dependent processes, but have also been shown to be blocked by selective σ -1 receptor antagonists.^[40] Therefore, σ -1 receptors regulating cognitive function may involve an interaction between neurosteroids and NMDA receptors. A recent study demonstrated that there is a general trend toward lower levels of neurosteroids in different brain regions of individuals with Alzheimer's disease or aged normal subjects. The metabolism of PB-008 is also shown to decrease in these subjects.^[106] A study using post-mortem brains showed a 26% reduction of [^3H]DGTG binding in the CA1 region of the hippocampi in patients affected by Alzheimer's disease.^[107] Some clinical studies suggest that hormone replacement therapy using estrogen and PB-008 improves cognitive function and may protect against age-associated memory decline.^[106,108-110] Thus, although the relationship between the σ -1 receptor

and dementia is unclear at present, σ -1 receptor agonists may improve memory and cognition in certain types of dementia.

2.2 Depression and Anxiety

2.2.1 Depression

The effects of σ -1 receptor ligands in animal models of depression have recently been investigated. The immobility time in the mouse forced swimming test was dose-dependently reduced by σ -1 receptor agonists.^[73] Structurally diverse σ receptor agonists such as (+)pentazocine, SA-4503 and igmesine (JO-1784) all decreased the immobility time in the test.^[73,74,111] This action was blocked by the σ -1 receptor antagonist NE-100.

Neurosteroids such as pregnenolone sulphate and PB-008 act as σ -1 receptor agonists, although progesterone acts apparently as a σ -1 receptor antagonist.^[3,40,47] Since sulphated neurosteroids act as reservoirs for the unsulphated neurosteroids in the brain, their levels are much higher than those of unsulphated forms.^[33] Pregnenolone sulphate and PB-008 have higher affinities for the σ -1 receptor than their unsulphated forms.^[32] Therefore, their concentrations in the brain may reach a sufficient level to interact with σ -1 receptors under certain psychological states such as stress, anxiety and aggression.^[33] The interaction between the σ -1 receptor and neurosteroids has been shown in the forced swimming test.^[73,74,111] The above neurosteroids significantly decreased the immobility time in these studies, an effect that was blocked by NE-100 and BD-1047, suggesting that certain neurosteroids exert antidepressant actions by interacting with σ -1 receptors.^[73,74]

Extract of the flowering plant St John's wort (*Hypericum perforatum*) is prescribed as an antidepressant in Germany. However, the mechanism of the antidepressant action of St John's wort remains unknown. Raffa^[112] tested the binding of hypericin (a major component of St John's wort) to 30 receptors or uptake sites that are suspected to be involved in the action of clinically used antidepressants. At 1.0 $\mu\text{mol/L}$, hypericin inhibited less than 40% of specific binding at all receptors or uptake sites except acetylcholine muscarinic receptors and σ receptors.^[112] Hypericine (1.0 $\mu\text{mol/L}$) inhibits 48% of

[³H]DTG binding. Because the author used subtype-nonspecific ligands in his assay, it is unclear at present which subtype of σ receptor (1 or 2) binds to hypericine. The antidepressant action of hypericin in the forced swimming test was inhibited by a σ -1 receptor antagonist.^[75] Bennett et al.^[113] systematically evaluated all articles on hypericin identified from a database and suggested that the σ receptor is a potential site of action for hypericin.

Because the pharmacological effect of σ -1 receptor ligands is typically modulatory, it is reasonable to propose developing compounds that possess a high affinity for both σ -1 receptors and other receptors that are known to be related to certain pathological states such as depression. A newly synthesised antidepressant OPC-14523 has a high affinity for both σ -1 receptors (47–56 nmol/L) and serotonin 5-HT_{1A} receptors (2.3 nmol/L).^[76] A single oral administration of OPC-14523 produced a marked antidepressant-like effect in the forced swimming test, whereas fluoxetine and imipramine required at least four days of repeated administration to show the same activity.^[76] This potent antidepressant-like action of OPC-14523 can be mimicked by combined administration of σ -1 and 5-HT_{1A} receptor agonists.^[77]

2.2.2 Anxiety

σ -1 ligands also exert anxiolytic action in animal behavioural tests.^[78] Animals exhibit a marked suppression of motility when they are placed in the same environment in which they had previously received an electric footshock (conditioned fear stress). (+)SKF-10047 reversed the conditioned fear stress and this effect was blocked by NE-100.^[78] However, this anxiolytic action was not seen with (+)pentazocine and DTG.

Gue et al.^[79,80] reported that the σ -1 receptor agonist igmesine attenuates an increase of colonic spike bursts induced by conditioned fear stress or corticotropin releasing hormone. Their findings are relevant with regard to gastrointestinal tract disorders that are frequently seen in anxiety and mood disorders.

More studies using structurally different σ -1 ligands may confirm the possible involvement of the σ -1 receptor in anxiety.

2.3 Psychosis, Drug-Dependence and Analgesia

2.3.1 Psychosis

Since σ -1 receptor ligands were originally expected to represent a new class of antipsychotics, a substantial number of experiments have been performed to examine the potential involvement of σ -1 receptors in schizophrenia using animal models. Amphetamine and PCP are frequently employed as the animal model of schizophrenia. Amphetamine mimicks acute positive symptoms of schizophrenia, such as delusion and hallucination. Methamphetamine and apomorphine are also known to induce similar effects. Because PCP causes not only acute psychosis mimicking positive symptoms but also long-lasting psychosocial dysfunction similar to the negative symptoms of schizophrenia, PCP-induced psychosis is suggested to be a suitable model for schizophrenia.^[114]

Some putative σ -1 receptor antagonists (e.g. BMS-181100 [BMX-14802]) inhibit methamphetamine-induced locomotor activity and apomorphine-induced stereotypy.^[115] However, highly selective σ -1 antagonists such as NE-100 and MS-377 do not affect locomotor activity induced by an injection of amphetamine.^[24,25,81] Some of these experiments used σ receptor ligands at high doses (>20 mg/kg);^[24] therefore, the inhibitory effect seen on psychostimulant-induced locomotor activity may not be solely due to interaction with the σ -1 receptor. In fact, the action of σ -1 ligands on amnesia (see section 2.1) is usually seen at doses below 1 mg/kg, and the effect usually disappears at higher doses (> 3 mg/kg).^[70] Selective antagonism of dopamine D₂ receptors seems to be more effective in attenuating locomotion and stereotypy induced by the acute administration of psychostimulants. Taken together, the data on the direct involvement of σ -1 receptors in schizophrenia, especially acute psychotic symptoms, is equivocal.

NE-100 is highly selective for σ -1 receptors and is the most well-defined functional σ -1 receptor antagonist.^[24] NE-100 does not affect behaviours induced by amphetamine, but inhibits several types of behaviour induced by PCP.^[24] The order of inhibiting potencies on PCP-induced head weaving by σ -1 receptor antagonists (NE-100 > haloperidol >

BMS-181100 > Dup-734) correlates well with their affinities for σ -1 receptors.^[24] PCP-induced behaviour is insensitive to selective D₂ receptor antagonists, but can be attenuated by very low doses of NE-100 (<0.1 mg/kg).^[24] In addition, NE-100 alone did not cause any extrapyramidal symptoms. Selective σ -1 receptor agonists such as (+)pentazocine, at low doses that caused no behavioural activity, enhanced the behavioural effect of dizocilpine, and this enhancement was blocked by NE-100 (0.01–0.1 mg/kg).^[24] Thus, it is very likely that one potential site of action of the σ -1 receptor is the regulation of ion channels coupled to NMDA receptors. A dysfunction of glutamatergic transmission, specifically, in the frontal cortex is implicated in schizophrenia.^[114,116] σ -1 receptor ligands may thus affect certain schizophrenic symptoms that are related to a dysfunction of glutamatergic transmission.

It has been shown that the microinjection of σ receptor ligands, including DTG and (+)SKF-10047, into the red nucleus causes dystonia in animals.^[117] Antipsychotics lacking affinity for σ receptors such as clozapine fail to induce this movement disorder. Interestingly, the microinjection of these σ ligands into the substantia nigra produces vigorous contralateral circling behaviour at extremely low doses. These data suggest the possibility that σ receptors are involved in the motor adverse effects of antipsychotics. The σ ligand-induced movement disorders seem to involve both σ -1 and σ -2 subtypes.^[85] This information will be important when the safety of σ -1 receptor ligands is evaluated in clinical trials.

2.3.2 Drug Dependence

Recently, the involvement of the σ -1 receptor in the pharmacological action of cocaine has been intensively investigated. Because cocaine has a moderate affinity for σ -1 receptors,^[118] it is suggested that certain actions of cocaine, inducing its toxic effects, may be attributed to its direct interaction with σ -1 receptors.^[99]

Matsumoto and colleagues^[86-88] clearly demonstrated that novel σ receptor antagonists at low doses (1 mg/kg) significantly inhibit convulsions and lethality induced by a toxic dose of cocaine. The toxicity of cocaine was potentiated by σ -1 receptor

agonists, indicating the potential involvement of σ -1 receptors in cocaine-induced toxicity.^[88]

Recently, the σ receptor antagonists BD-1047 and LR-172 were shown to inhibit cocaine-induced locomotor activity.^[86] Both compounds possess similar high affinities for σ -1 receptors. However, LR-172, which possesses much greater affinity at σ -2 receptors, was a more potent inhibitor than BD-1047 against cocaine-induced locomotor activity.^[86] These data may suggest the involvement of σ -2 receptors in this action.

The affinity of cocaine for σ -1 receptors is about 2 μ mol/L.^[118] The intracellular concentration of cocaine in the brain of cocaine abusers might not reach a sufficient level to occupy σ -1 receptors on the ER. Romieu et al.^[84] reported that conditioned place preference induced either by cocaine or a selective dopamine transporter inhibitor without any σ -1 receptor affinity is inhibited by a selective σ -1 receptor antagonist, suggesting that σ -1 receptors may play a role downstream of dopaminergic transduction. Further studies are required to clarify the mechanism by which σ -1 receptors may be involved in cocaine abuse.

Ujike et al.^[82,83] reported that putative σ receptor antagonists, at doses *per se* not affecting spontaneous locomotor activity, block the development of behavioural sensitisation induced by cocaine and methamphetamine. This action of σ receptor antagonists was confirmed by other researchers using the selective σ -1 antagonist MS-377.^[81]

2.3.3 Analgesia

Several reports indicate that σ -1 receptor agonists can inhibit opioid-induced analgesia.^[89-91] As shown in figure 1, σ -1 receptors are enriched in the spinal cord and peripheral nerves where opioid receptors are enriched. σ -1 receptors and ligands may thus regulate opiate receptor activities at these loci.

3. Potential Therapeutic Targets of Selective σ -1 Receptor Ligands

3.1 Schizophrenia

The demonstration that benzomorphans, such as SKF-10047, produce 'canine delirium' and psychosis in dogs^[1] led to the proposal that σ -1 receptors mediate these effects. Binding studies using

[³H]SKF-10047 and (+)[³H]-3-PPP identified an abundance of σ receptors in the brain.^[7,10-13] An important role of σ receptors in schizophrenia is also suggested by the high affinity of many established antipsychotic drugs for σ receptors.^[7] However, in the early reports on benzomorphan-induced psychosis in humans, very high dose of pentazocine were usually administered.^[5,6] In most case, a mixture of both (+) and (-) isomers were given.^[5,6] Considering that these benzomorphans have moderate to high affinities for the PCP site,^[9] the psychotomimetic action of benzomorphans might be mainly attributed to their interaction with the PCP site. In fact, recently synthesised highly selective σ -1 receptor agonists do not have any psychotomimetic activity in animal studies,^[76,102] negating a direct relationship between activation of σ -1 receptors and acute psychosis. Binding assays using post-mortem brains of schizophrenic patients^[119,120] and association studies of polymorphism of σ -1 receptor genes in schizophrenic patients^[121,122] have been performed. However, results vary and are not conclusive.

Some σ receptor ligands have been tested in clinical trials (see table I). Rimcazole (BW-234U) was the first σ receptor ligand to be tested in a clinical trial. After a 1-week placebo washout period, rimcazole was administered for 4 weeks to 11 patients with schizophrenia who were experiencing an acute exacerbation of symptoms.^[27] Subjects were assigned on a double-blind, randomised basis to one of four flexible dosing treatment regimens (rimcazole 20–80 mg/day, rimcazole 100–400 mg/day, chlorpromazine 400–1600 mg/day or placebo). Rimcazole was not associated with any significant improvement in the Brief Psychiatric Rating Scale (BPRS) and Clinical Global Impressions (CGI) scores. However, it did not show any neurological adverse effects. Although rimcazole selectively binds to σ -1 receptors, it is a weak antagonist (inhibition constant [K_i] = 2.4 μ mol/L).^[24] In contrast to these results, in earlier open studies, rimcazole was found to cause improvement of negative symptoms such as depression and anergia, but a worsening of acute positive symptoms.^[123,124]

BMS-181100, a relatively selective and potent σ -1 receptor antagonist, was also tested in patients with acute exacerbation of schizophrenic symptomatology. Subjects were included in this study if their

BPRS scores were ≥ 35 . In a single-blind trial, treatment with BMS-181100 (500–1500mg twice daily up to a maximum of 3000 mg/day) for 4 weeks in 28 patients was not associated with any improvements in BPRS and CGI score.^[28] The most frequent adverse effect was mild to moderate sinus tachycardia. Elevations in creatine phosphokinase (CPK) levels, agitation and insomnia were seen in some patients.

There are a number of issues associated with these two trials^[27,28] that need to be considered: (i) the functional activity of the ligands and their selectivity for the σ -1 receptor were not fully characterised via *in vivo* and *in vitro* assays; (ii) only schizophrenic patients in the acute psychotic phase were studied; (iii) the subclass of symptoms was assessed only using the BPRS, which mainly reflects the degree of positive symptoms; (iv) the duration of evaluation might not have been long enough (evaluation longer than 4 weeks is usually required for examining changes in negative symptoms); and (v) an effective dose might not have been used in the BMS-181100 study^[28] (too high in this trial).

Modell et al.^[31] tested the effect of eliprodil (SL-82.0715), a potent σ -1 receptor ligand, on schizophrenia in an open-label, dose-ranging study. This compound exacerbated acute psychotic symptoms in some patients in an earlier study,^[125] as was seen in the rimcazole and BMS-181100 studies.^[27,28,123,124] Therefore, subjects with the diagnosis of residual-type schizophrenia with a predominant negative symptomatology (score of >28 on the negative subscale of the Positive and Negative Syndrome Scale [PANSS]) were included in this study. After a 1- to 2-week washout period, patients received eliprodil for 4 weeks (2.5 mg/day for the first week, then up to 75 mg/day). Although the number of subjects was limited (n = 10), a significant reduction of scores in negative symptoms, but not in positive symptoms, according to the PANSS was found.

Panamesine (EMD-57445), a potent σ receptor ligand with antidopaminergic activity, was shown to improve both positive and negative symptoms in an open clinical trial.^[29,30] In this study, panamesine (15mg on day one, 30mg on day two, 45mg on day three and 60mg from day four onwards) was administered to 12 schizophrenic patients for 4 weeks, and effects were assessed by both the BPRS and the PANSS. Statistical analyses showed a significant

reduction of PANSS scores related to both positive and negative symptoms after 3 weeks of panamesine use. However, panamesine induced mild dyskinctic movements in 5 of the 12 patients. It needs to be further characterised whether these effects, or a part of them, are mediated by a direct interaction with σ -1 receptors, because a metabolite of the drug has potent antidopaminergic activity.^[126]

Taken together, these results suggest that selective σ -1 receptor antagonists appear to have therapeutic effects on some symptoms of schizophrenia but not acute positive symptoms. As mentioned in section 2.3, selective σ -1 receptor antagonists may not affect acute psychosis mediated by the activation of dopaminergic transduction, but can inhibit PCP-induced behaviours in animals. So far, no anti-psychotic which manipulates glutamatergic neural transmission is available. Therefore, the beneficial effect of σ -1 receptor ligands might be seen when they are used to target symptoms of schizophrenia that are mediated predominantly via glutamatergic systems rather than dopaminergic systems. Thus, σ -1 receptor ligands may affect negative symptoms of schizophrenia, which are hypothesised to be mediated, at least partly, by glutamatergic transmission.

At low doses, psychostimulants exacerbate psychosis in patients with remitting schizophrenia. Long-term drug abusers sometimes show psychotic symptoms similar to those seen in schizophrenia when taking a low dose of psychostimulants or even when stressed. Therefore, the behavioural sensitisation to psychostimulants has been used as a model of schizophrenia, especially for studies of deterioration and exacerbation of manifested symptoms.^[127] Judging from the behavioural sensitisation data in animals,^[81-83] σ -1 receptor antagonists might have a prophylactic effect in preventing the recurrence of schizophrenia. However, so far, no clinical study regarding this issue has been performed.

3.2 Depression and Senile Dementia

It has been established that abnormalities of serotonergic and noradrenergic neural transmission are involved in the pathophysiology of depression. However, recent studies have also demonstrated the involvement of neurosteroids in depression.^[36] Allopregnenolone and PB-008, in particular, are of

significant importance in depression.^[36,37] Several studies have also suggested the involvement of neurosteroids in premenstrual dysphoric disorder (PMDD)^[128] and attention-deficit hyperactivity disorder.^[129]

In patients with major depression, neurosteroid levels in the CSF are lower than in control subjects and these levels normalise after successful treatment with antidepressants.^[36,37] SSRIs have been shown to normalise levels of allopregnenolone in a serotonin-independent manner.^[37] It is well known that plasma cortisol levels are increased in depressed patients,^[36,130] and that PB-008 regulates cortisol levels.^[131] Some studies found significantly higher cortisol/PB-008 ratios in depressed patients compared with controls.^[36,132] Administration of PB-008 has been shown to reduce plasma cortisol levels.^[132]

The important neurosteroid-targeted receptors in the brain include the GABA_A, NMDA and σ -1 receptors.^[33,36] PB-008 interacts with σ -1 receptors as an agonist and exerts antidepressant-like action in an animal model of depression.^[73,74] The molecular mechanism underlying this action of σ -1 receptors is unclear at present. However, it is very likely that the σ -1 receptor plays an important role in the biological action of neurosteroids (or neuroactive steroids) in depression. Orally administered neuroactive steroids may not result in an increase in levels in the brain due to a rapid metabolism. Selective σ -1 receptor agonists may act in a complementary fashion to neurosteroids in the brain.

So far, the only reports of the clinical use of a σ -1 receptor ligand as an antidepressant are of igmesine. In an open trial,^[26] igmesine showed a significant improvement in 31 severely depressed inpatients. A double-blind placebo-controlled study comparing igmesine 25 and 100 mg/day with fluoxetine 20 mg/day in 348 patients with major depressive disorder was performed in three different countries (UK, Poland and the Czech Republic). Although no significant effect was found comparing total samples, a subset of patients receiving igmesine 25 mg/day from UK sites ($n = 263$) showed a 3 point greater decrease in total Hamilton Depression Rating Scale score than the placebo group ($p < 0.05$).^[26]

To date, there have been no reports of the use of a σ -1 receptor ligand to treat dementia. According to aforementioned recent preclinical studies, however,

it is highly possible that σ -1 receptor agonists may augment cognitive functions and improve mood in patients with psychiatric disorders. Because the action of σ -1 receptor ligands is mainly modulatory, σ -1 receptor ligands are expected to restore the existing (but functionally declining) neural transmission.

4. Conclusion

Because the σ -1 receptor is involved in higher-ordered brain functions such as memory, cognition and mood, it may be involved in the development of neuronal plasticity. The pathophysiology of certain neuropsychiatric disorders, such as drug dependence and depression, has been shown to involve neural plasticity that may be related to dendrite outgrowth and synapse formation.^[92,133-135] Thus, σ -1 receptor ligands might show therapeutic actions by affecting the remodelling of neural membranes, as we have recently proposed.^[54,55,64,99]

A unique pharmacological feature of selective σ -1 receptor ligands is their 'modulatory' action. For example, in *in vitro* studies, σ -1 receptor ligands did not affect intracellular Ca^{2+} levels by themselves, but potentiated IP3-induced Ca^{2+} mobilisation.^[47] In behavioural studies examining mnemonic processes, σ -1 receptor agonists showed effects only when memory was impaired.^[70]

We do not know exactly how σ -1 receptor ligands exert this modulatory action, but we propose here a hypothetical scheme of this action (figure 2). When dopamine receptor ligands are administered to normal animals, agonists *per se* induce physiological or behavioural alterations by activating the receptors. Conversely, antagonists induce certain actions by displacing endogenous dopamine. These actions are not seen with σ -1 receptor ligands. Both σ -1 receptor agonists and antagonists apparently show no effects when administered to normal animals (see section 2.1). σ -1 receptor proteins *per se* may possess certain unknown biological activity, even in the absence of ligands. However, the existence of endogenous σ -1 receptor ligands does not explain the existing biological activity of σ -1 receptors, because σ -1 receptor antagonists lack pharmacological effects in normal subjects. σ -1 receptor ligands may merely work as a modulator of the existing activity of σ -1 receptors. When σ -1 recep-

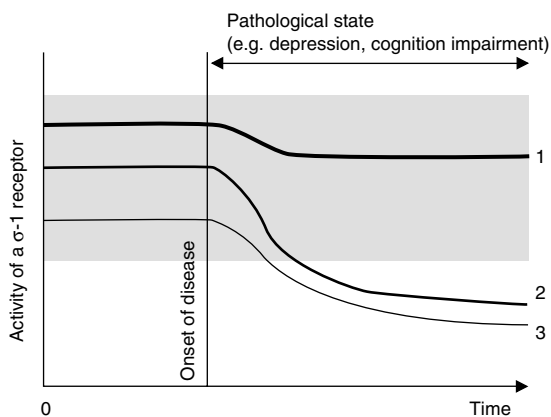


Fig. 2. A hypothetical scheme of the 'modulatory' action of σ -1 receptor ligands. σ -1 receptors may possess innate biological activity. In the normal brain, the activity of σ -1 receptors would be maintained within a sufficient range (shaded area) for regulating neurophysiological functions. The factor that maintains the activity of σ -1 receptors is unknown at present. If neuronal activity decreases due to certain pathological states or influences from exogenous compounds, σ -1 receptors would become hypoactive (line 2). σ -1 receptor agonists at appropriate doses may act as modulators to compensate for the reduced activity of the receptor (line 1). σ -1 antagonists would eliminate the augmentation of the σ -1 receptor activity induced by σ -1 agonists (line 3). At excessive doses of an agonist, the effects of σ -1 receptors could disappear (shifting from line 1 to 2) as has been shown in *in vivo* and *in vitro* studies.^[39,47,65]

tors become hypoactive (or hyperactive) under certain pathological states, σ -1 ligands might compensate for (or normalise) the biological activity of the protein. This hypothesis should be further addressed in the future.

Potential therapeutic targets of σ -1 ligands may include depression and senile dementia. The action of σ -1 ligands in these diseases might be, at least in part, related to an effect on neurosteroids via an unknown mechanism. The effects of σ -1 receptor ligands in schizophrenia seem to be equivocal, although their effects on certain symptoms (e.g. negative symptoms, depressive symptoms and prophylaxis) warrant further investigation.

Because of their modulatory action, σ -1 ligands may be active only in patients who have neuropsychiatric diseases. Therefore, they might be expected to show only minimal adverse effects. Also because of their unique action, it is reasonable to propose developing compounds that possess a high affinity for both σ -1 receptors and other receptors

which are known to be involved in certain pathological states such as depression.

Acknowledgements

The authors are supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, USA. The authors have no financial interest that may confound the publication of this article.

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