Identification of the Sigma-2 Receptor: Distinct from the Progesterone Receptor Membrane Component 1 (PGRMC1)

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Editorial

The sigma receptor (σR) subtypes, σ1 and σ2, have been mischaracterized [1,2]. A recent study suggested that the σ2R is the progesterone receptor membrane component 1 (PGRMC1) in rat livers. This finding was supported by the use of a novel photo affinity probe for σ2Rs, 5-[3-(4-[4azido2(4[6,7dimethoxy3,4dihydroisoquinolin 2(1H)y1]butylcarbamoyl]phenoxy]butyl)thioureido]-2-(6-hydroxy-3-oxo-3H-xanthen-9-l)benzoic acid (WC-21) [3]. Since that study, many have accepted that these two entities are the same. More recent studies have, however, indicated that this identification was mischaracterized [4,5]. This mischaracterization is significant for the establishment of σ2R pharmacology. Precise pharmacological characterization of the σ2R is important because it has been implicated with stimulant abuse [6,7].

σRs are unique intracellular chaperone proteins [8] initially thought to be opioid receptor subtypes [9]. They have been classified into two subtypes based on specific radioligand binding assays using [3H](+)-pentazocine for σ1Rs and [3H]1,3-di-o-tolylguanidine ([3H]DTG, in the presence of dextrorlrorphan to mask the σ1R) for σ2Rs in rat liver and kidney membranes [10]. Currently, the more selective σ1R ligand (+)-pentazocine has replaced dextrorrlophorpharan to mask the σ1R [7,11-14]. The σ1R has already been cloned as a 25-29 kDa chaperone protein composed of 223 amino acids [4,8,15]. It is widely distributed throughout the body [16-20]. Upon binding with agonists or under cellular stress, σ1Rs translocate from their primary endoplasmic reticulum (ER) location to different subcellular compartments where they can regulate ion channels and G-protein-coupled-receptor (GPCR) signaling [8,21-24]. In vivo functional studies on σ1Rs suggest that they play a substantial role in various cellular functions. Drugs acting at this receptor have been studied for their potential therapeutic effects in cancer, human immunodeficiency virus (HIV) infection, various psychiatric disorders, and substance abuse [1,25].

The σ1R is not a GPCR. Thus, it is challenging to determine what constitutes an agonist or antagonist. For example, in vitro studies using NG-108 and Chinese Hamster Ovary...
(CHO) cells have demonstrated that the selective σ₁R ligands PRE-084 and (+)-pentazocine can dose-dependently cause the dissociation of σ₁R from a binding immunoglobulin protein/78 kDa glucose-regulated protein (BiP/GRP-78), another ER chaperone [8,26]. Thus, they serve as agonists. In contrast, the σ₁R ligands haloperidol and 4-methoxy-3-(2-phenylethoxy)-N,N-dipropylbenzeneethanamine (NE-100) alone do not affect the σ₁R-BiP association but both completely inhibit the dissociation of σ₁R from BiP caused by (+)-pentazocine: they serve as antagonists [8,26]. In vivo, however, there is--as yet--no established functional assay for the σR subtypes. However, there is evidence showing a dose-dependent antagonism in vivo using the in vitro σ₁R antagonists against the in vitro σ₁R agonists using drug self-administration procedures [7,12,27,28]. Thus, it appears that the in vitro agonist-antagonist relationship will apply some in vivo responses.

The [³H](+)-pentazocine-inaccessible σR, the σ₂R, is an 18-21 kDa protein that has not been cloned yet [3,20,29-31]. However, a previous study using the radioligands [³H](+)-pentazocine, and [³H]DTG (in the presence of dextralorphan) and a Flotillin-2 dotblotting technique in rat liver membranes found that σ₂Rs are primarily localized in membrane lipid rafts whereas the σ₁R localization appears in both raft and non-raft membrane domains [32]. The σ₁R is dynamic and can translocate from its primary ER location to different subcellular compartments [24]. Previous mass spectrometry studies identified the σ₂R-like proteins as being dimers consisting of H2A/H2B, the human nucleosomal proteins [33,34], which were defined using [³H]-1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetra-hydronaphthalen-1-yl)propyl]piperazine ([³H]PB28) as a radioligand having a 19-fold higher affinity for the σ2 than for the σ₁ receptors [35]. Abate et al. [34] showed that [³H]PB28 accumulation was up to five-fold higher in nuclear fractions than in cytosolic fractions in SK-N-SH and MCF7 cells. However, the dimer differs from the σ₂R in membrane association [32]. Thus, the identity of σ₂Rs as nucleosomal proteins does not appear to be viable.

Due to the lack of a known σ₂R amino acid sequence, photoaffinity labeling remains the most viable approach for visualizing the receptor using sodium dodecyl sulfate (SDS) gels [29]. The basic principle is to covalently combine a photoactivatable σ₂R-binding probe with the receptor such that the probe (radioactive- or fluorescent-labeled) remains with the protein even after denaturation with SDS [29]. Using a novel photoaffinity probe for σ₂Rs, WC-21, a recent study identified the σ₂R as the PGRMC1 in rat livers [3]. For example, the non-selective σ₁/2R ligand DTG prevented the photolabeling of PGRMC1 (with WC-21) [3]. Further, an immunocytochemical study revealed that both PGRMC1 and (1R,3r,5S)-9-(10-[(7-Nitrobenzo[c] [1,2,5]oxadiazol-4-yl)amino]decyl)-9 azabicyclo[3.3.1]nonan-3-yl (2-methoxy-5-methylphenyl) carbamate (SW120), a fluorescent σ₂R ligand, colocalize with molecular markers of the ER and mitochondria in HeLa cells [3]. As noted for the σ₁R, studies utilizing various in vitro techniques indicated that σ₂Rs are intracellular proteins. However, the affinity of DTG for the PGRMC1 was not reported in the study [3]. Nonetheless, it appears that the identification of the σ₂R as the PGRMC1 [3] has been accepted widely. However, two recent studies [1,2] demonstrated a more viable data set against this identification as follows:

1. The molecular size of PGRMC1 (25 kDa) is approximately 7 kDa higher than that of the σ₂R (∼18 kDa) [4].
2. Using specific photolabeling with [125I]-iodoazido-fenpropimorph ([125I]-IAF), the photolabeled \( \sigma_2 \)R band was not diminished in NSC34 cells devoid of or overexpressing the PGRMC1 [4]. Further, PGRMC1 knockout did not reduce [125I]-IAF photolabeling of the \( \sigma_2 \)R (18-21 kDa band) that was protectable by DTG and the highly \( \sigma \)-R-selective CM compounds [e.g. 1-(4-[6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]butyl)-3-methyl-1H-benzo[d]imidazo[2,3H]-one hydrochloride (CM 398)] [4]. The lack of influence of PGRMC1 knockout on the photolabeling of \( \sigma_2 \)R indicates a lack of a \( \sigma_2 \)R ligand-binding pocket formed by PGRMC1/\( \sigma_2 \)R complexes. The results also suggest that the \( \sigma_2 \)R is not a splice variant of the PGRMC1, thus, these two proteins are derived from different genes.

3. Alternatively, the PGRMC1 may be another DTG-binding protein that does not bind the photoprobe [125I]-IAF. If PGRMC1 is a high-affinity DTG binding site, elevation of PGRMC1 protein levels would result in an increase in maximal binding of \([^3H]\)DTG. However, neither the Bmax nor Kd values for \([^3H]\)DTG changed significantly in response to PGRMC1 overexpression, knockout or silencing in NSC34 cells [4] or human MCF7 adenocarcinoma cell lines [5] which are devoid of the \( \sigma_1 \)R [36].

4. Progesterone has been reported to be a high-affinity (Kd=35 nM) ligand for PGRMC1 (Table 1). However, the Ki value of progesterone for the \( \sigma_2 \)R [4] is approximately 406-fold higher than the Kd value for PGRMC1 in rat liver membranes (Table 1). Further, the Ki value of DTG for the PGRMC1 is 472,000 ± 420,000 nM (Table 1) using cold (+)-pentazocine to block the \( \sigma_1 \)R [4], which is approximately 15,000-fold higher than that for the \( \sigma_2 \)R [4] (Table 1). However, the Ki value of DTG for the PGRMC1 [4] was shown to be >1,000-fold lower than that obtained in a previous study [37] (Table 1). This discrepancy likely results from the lack of use of a selective cold blocker at the \( \sigma_1 \)R in the previous study [37] since DTG can also bind the \( \sigma_1 \)R with high affinity (Table 1). The binding profile of DTG for the PGRMC1 has been consistent with that for haloperidol, another non-selective \( \sigma_1/2 \)R ligand [4] (Table 1). Thus, the PGRMC1 is not a high-affinity DTG binding site, which also means that the PGRMC1 is not the \( \sigma_2 \)R.

Together, these new data [4,5] clearly suggest that the \( \sigma_2 \)R and PGRMC1 are two different molecular entities. Furthermore, the photo affinity probe containing a \( \sigma_2 \)R-directing moiety that led to the identification of PGRMC1 [3] as the \( \sigma_2 \)R (with WC-21), likely binds both \( \sigma_2 \)R and PGRMC1. The identification of the \( \sigma_2 \)R as distinct from the PGRMC1 [4,5] should have considerable impact especially in the cancer study field since the \( \sigma_2 \)R has been developed as a biomarker for various tumor cells [38]. Other studies have attempted to examine the correlation between the binding affinity of various \( \sigma \)R ligands and their ability to produce effects both \textit{in vitro} and \textit{in vivo} through the \( \sigma_2 \)R [35,39]. However, the evidence for \( \sigma_2 \)R-mediated actions from these studies is not compelling because of the mixed use of \( \sigma \)R agonist-like and antagonist-like ligands. Thus, the pharmacology and physiological role of \( \sigma_2 \)Rs remain undetermined due to unsuccessful efforts to clone the receptor and a lack of selective ligands. On the other hand, \textit{in vitro} functional studies have demonstrated that the
activation of the $\sigma_2$R resulted in the synthesis and release of dopamine in the rat brain [6,7]. Thus, future studies that further explore $\sigma_2$R pharmacology may result in a better understanding of the dopamine-mediated reinforcing mechanism associated with stimulant abuse and other dopamine-related diseases (e.g. Parkinson’s disease and schizophrenia).

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References

1. Katz JL, Su TP, Hiranita T, Hayashi T, Tanda G, et al. A role for sigma receptors in stimulant self-administration and addiction. Pharmaceuticals. 2011; 4:880–914. [PubMed: 21904468]
2. Katz JL, Hong WC, Hiranita T, Su TP. A role for sigma receptors in stimulant self-administration and addiction. Behav Pharmacol. 2016; 27:100–115. [PubMed: 26650253]
3. Xu J, Zeng C, Chu W, Pan F, Rothfuss JM, et al. Identification of the PGRMC1 protein complex as the putative sigma-2 receptor binding site. Nature communications. 2011; 2:380.
4. Chu UB, Mavlyutov TA, Chu ML, Yang H, Schulman A, et al. The sigma-2 receptor and progesterone receptor membrane component 1 are different binding sites derived from independent genes. EBioMedicine. 2015; 2:1806–1813. [PubMed: 26870805]
5. Abate C, Niso M, Infantino V, Menga A, Berardi F. Elements in support of the ‘non-identity’ of the PGRMC1 protein with the $\sigma_2$ receptor. Eur J Pharmacol. 2015; 758:16–23. [PubMed: 25843410]
6. Weiser SD, Patrick SL, Mascarella SW, Downing-Park J, Bai X, et al. Stimulation of rat striatal tyrosine hydroxylase activity following intranigral administration of $\sigma$ receptor ligands. Eur J Pharmacol. 1995; 275:1–7. [PubMed: 774655]
7. García-Ramírez L, Green JL, Hiranita T, Kopajtic TA, Mereu M, et al. Sigma receptor agonists: receptor binding and effects on mesolimbic dopamine neurotransmission assessed by microdialysis. Biological Psychiatry. 2011; 69:208–217. [PubMed: 20950794]
8. Hayashi T, Su TP. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca2+ signaling and cell survival. Cell. 2007; 131:596–610. [PubMed: 17981125]
9. Martin W, Eades CG, Thompson J, Huppler RE, Gilbert PE. The effects of morphine-and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. J Pharmacol Exp Ther. 1976; 197:517–532. [PubMed: 945347]
10. Hellewell SB, Bruce A, Feinstein G, Orringer J, Williams W, et al. Rat liver and kidney contain high densities of sigma 1 and sigma 2 receptors: characterization by ligand binding and photoaffinity labeling. Eur J Pharmacol. 1994; 268:9–18. [PubMed: 7925616]
11. Hiranita T, Soto PL, Kohut SJ, Kopajtic T, Cao J, et al. Decreases in cocaine self-administration with dual inhibition of the dopamine transporter and $\sigma$ receptors. J Pharmacol Exp Ther. 2011; 339:662–677. [PubMed: 21859929]
12. Hiranita T, Mereu M, Soto PL, Tanda G, Katz JL. Self-administration of cocaine induces dopamine-independent self-administration of sigma agonists. Neuropsychopharmacology. 2013; 38:605–615. [PubMed: 23187725]
13. Hiranita T, Soto PL, Tanda G, Kopajtic TA, Katz JL. Stimulants as specific inducers of dopamine-independent $\sigma$ agonist self-administration in rats. J Pharmacol Exp Ther. 2013; 347:20–29. [PubMed: 23908387]
14. Hiranita T, Wilkinson DS, Hong WC, Zou MF, Kopajtic TA, et al. 2-isooxazol-3-phenyltropane derivatives of cocaine: molecular and atypical system effects at the dopamine transporter. J Pharmacol Exp Ther. 2014; 349:297–309. [PubMed: 24518035]
15. Hanner M, Moebius FF, Flandorfer A, Knaus HG, Striessnig J, et al. Purification, molecular cloning, and expression of the mammalian sigma1-binding site. Proc Natl Acad Sci U S A. 1996; 93:8072–8077. [PubMed: 8755605]

16. Hayashi T, Justinova Z, Hayashi E, Cormaci G, Mori T, et al. Regulation of σ-1 receptors and endoplasmic reticulum chaperones in the brain of methamphetamine self-administering rats. J Pharmacol Exp Ther. 2010; 332:1054–1063. [PubMed: 19940104]

17. Walker JM, Bowen WD, Goldstein SR, Roberts AH, Patrick SL, et al. Autoradiographic distribution of [3H](+)-pentazocine and [3H] 1,3-di-o-tolylguanidine (DTG) binding sites in guinea pig brain: a comparative study. Brain Res. 1992; 581:33–38. [PubMed: 1323368]

18. Nguyen VH, Kassiou M, Johnston GA, Christie MJ. Novel subtype-selective trishomocubanes. Eur J Pharmacol. 1996; 311:233–240. [PubMed: 8891604]

19. Inoue A, Sugita S, Shoji H, Ichimoto H, Hide I, et al. Repeated haloperidol treatment decreases σ1 receptor binding but does not affect its mRNA levels in the guinea pig or rat brain. Eur J Pharmacol. 2000; 401:307–316. [PubMed: 10936488]

20. Hellewell SB, Bowen WD. A sigma-like binding site in rat pheochromocytoma (PC12) cells: decreased affinity for (+)-benzomorphans and lower molecular weight suggest a different sigma receptor form from that of guinea pig brain. Brain Res. 1990; 527:244–253. [PubMed: 2174717]

21. Cormaci G, Mori T, Hayashi T, Su TP. Protein kinase A activation down-regulates, whereas extracellular signal-regulated kinase activation up-regulates σ-1 receptors in B-104 cells: Implication for neuroplasticity. J Pharmacol Exp Ther. 2007; 320:202–210. [PubMed: 17050780]

22. Aydar E, Palmer CP, Klyachko VA, Jackson MB. The sigma receptor as a ligand-regulated auxiliary potassium channel subunit. Neuron. 2002; 34:399–410. [PubMed: 11988171]

23. Hong W, Werling LL. Evidence that the σ1 receptor is not directly coupled to G proteins. Eur J Pharmacol. 2000; 408:117–125. [PubMed: 11080517]

24. Hayashi T, Su TP. Intracellular dynamics of σ-1 receptors (σ1 binding sites) in NG108-15 cells. J Pharmacol Exp Ther. 2003; 306:726–733. [PubMed: 12730356]

25. Maurice T, Su TP. The pharmacology of sigma-1 receptors. Pharmacol Ther. 2009; 124:195–206. [PubMed: 19619582]

26. Hayashi T, Su TP. Regulating ankyrin dynamics: Roles of sigma-1 receptors. Proc Natl Acad Sci U S A. 2001; 98:491–496. [PubMed: 11149946]

27. Hiranita T, Soto PL, Tanda G, Katz JL. Reinforcing effects of σ-receptor agonists in rats trained to self-administer cocaine. J Pharmacol Exp Ther. 2010; 332:515–524. [PubMed: 19892920]

28. Hiranita T, Soto PL, Tanda G, Kopajtic TA, Katz JL. Stimulants as specific inducers of dopamine-independent σ agonist self-administration in rats. J Pharmacol Exp Ther. 2013; 347:20–29. [PubMed: 23908387]

29. Chu UB, Mavluytov TA, Chu ML, Yang H, Schulman A, et al. The σ2 receptor and progestrone receptor membrane component 1 are different binding sites derived from independent genes. EBioMedicine. 2015; 2:1806–1813. [PubMed: 26870805]

30. Bowen WD, Hellewell SB, McGarry KA. Evidence for a multi-site model of the rat brain σ receptor. Eur J Pharmacol. 1989; 163:309–318. [PubMed: 2542066]

31. Pal A, Hajipour AR, Fontanilla D, Ramachandran S, Chu UB, et al. Identification of regions of the σ-1 receptor ligand binding site using a novel photoprobe. Mol Pharmacol. 2007; 72:921–933. [PubMed: 17622576]

32. Gebreselassie D, Bowen WD. Sigma-2 receptors are specifically localized to lipid rafts in rat liver membranes. Eur J Pharmacol. 2004; 493:19–28. [PubMed: 15189760]

33. Colabufo NA, Berardi F, Abate C, Contino M, Niso M, et al. Is the σ2 receptor a histone binding protein? J Med Chem. 2006; 49:4153–4158. [PubMed: 16821775]

34. Abate C, Elenewski J, Niso M, Berardi F, Colabufo NA, et al. Interaction of the σ2 receptor ligand PB28 with the human nucleosome: computational and experimental probes of interaction with the H2A/H2B dimer. ChemMedChem. 2010; 5:268–273. [PubMed: 20074626]

35. Colabufo NA, Berardi F, Contino M, Perrone R, Tortorella V. A new method for evaluating σ2 ligand activity in the isolated guinea-pig bladder. Naunyn Schmiedebergs Arch Pharmacol. 2003; 368:106–112. [PubMed: 12879208]
36. Lee IT, Chen S, Schetz JA. An unambiguous assay for the cloned human sigma 1 receptor reveals high affinity interactions with dopamine D4 receptor selective compounds and a distinct structure–affinity relationship for butyrophenones. Eur J Pharmacol. 2008; 578:123–136. [PubMed: 17961544]

37. Meyer C, Schmieding K, Falkenstein E, Wehling M. Are high-affinity progesterone binding site(s) from porcine liver microsomes members of the σ receptor family? Eur J Pharmacol. 1998; 347:293–299. [PubMed: 9653896]

38. van Waarde A, Rybczynska AA, Ramakrishnan NK, Ishiwata K, Elsinga PH, et al. Potential applications for sigma receptor ligands in cancer diagnosis and therapy. Biochim Biophys Acta. 2015; 1848:2703–2714. [PubMed: 25173780]

39. Matsumoto RR, Pouw B. Correlation between neuroleptic binding to σ1 and σ2 receptors and acute dystonic reactions. Eur J Pharmacol. 2000; 401:155–160. [PubMed: 10924920]

40. McCann DJ, Su TP. Solubilization and characterization of haloperidol-sensitive (+)-[3H]SKF-10,047 binding sites (sigma sites) from rat liver membranes. J Pharmacol Exp Ther. 1991; 257:547–554. [PubMed: 1851829]

41. Northcutt AL, Hutchinson MR, Wang X, Baratta MV, Hiranita T, et al. DAT isn't all that: cocaine reward and reinforcement require Tolllike receptor 4 signaling. Mol Psychiatry. 2015; 20:1525–1537. [PubMed: 25644383]

42. Guo L, Zhao J, Jin G, Zhao B, Wang G, et al. SKF83959 is a potent allosteric modulator of sigma-1 receptor. Mol Pharmacol. 2013; 83:577–586. [PubMed: 23295385]

43. Peluso JJ, Romak J, Liu X. Progesterone receptor membrane component-1 (PGRMC1) is the mediator of progesterone's antiapoptotic action in spontaneously immortalized granulosa cells as revealed by PGRMC1 small interfering ribonucleic acid treatment and functional analysis of PGRMC1 mutations. Endocrinology. 2008; 149:534–543. [PubMed: 17991724]
Table 1

Inhibition (Ki values) by various compounds of specific binding to the σ1, σ2 receptors or PGRMC1. Values represent means ± SEM in nM. Values in parentheses are 95% confidence limits.

| Compound       | σ1R (26 kDa) [4] | σ2R (~18 kDa) [4] | PGRMC1 (25 kDa) [4]         |
|----------------|------------------|-------------------|----------------------------|
| (+)-Pentazocine| **63.9 (40)      | 224 (95% confidence limits: 195-257) [13] | **63.9 (40)                |
| DTG            | 57.4 (95% confidence limits: 49.3-66.7) [7] | *31.5 (SEM=3.3) [5] | 472,000 (SEM=420,000) [4] 310 [37] |
| Haloperidol    | 2.91 (95% confidence limits: 2.69-3.14) [41] | 31.5 (SEM=0.5) [4] | 350,000 (SEM=19,000) [4]   |
| Progesterone   | 1.540 (SEM=180) [42] | 14.200 (SEM=4.900) [4] | *35 [43]                  |

* Kd value
** IC50 values