Pergamon

Pharmacology Biochemistry and Behavior, Vol. 50, No. 4, pp. 587–592, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00346-7

Antinociception Following 1,3,-di-*o*-Tolylguanidine, a Selective σ Receptor Ligand

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Received 16 February 1994

KEST, B., J. S. MOGIL, W. F. STERNBERG, R. N. PECHNICK AND J. C. LIEBESKIND. *Antinociception following 1,3,-di-o-tolylguanidine, a selective σ receptor ligand.* PHARMACOL BIOCHEM BEHAV **50**(4) 587–592, 1995.– The role of σ receptors in antinociceptive processes remains equivocal, because previous σ drugs also bind to PCP/NMDA and opiate receptors. The present study examined the antinociceptive effects of the high-affinity, σ-selective ligand 1,3-di-*o*-tolylguanidine (DTG; 10, 15, and 20 mg/kg, IP) on tail-withdrawal latencies in mice. DTG produced significant but short-lived increases in withdrawal latencies at all dose levels. DTG also produced hypothermia, but this effect was dissociable from antinociception. The highly selective σ ligand rimcazole (10 and 25 mg/kg, IP) antagonized DTG antinociception in a dose-dependent manner. The opiate antagonist naloxone and the PCP/NMDA antagonist MK-801 were, however, without effect. Haloperidol, which also binds to σ receptors, increased withdrawal latencies but did not alter DTG antinociception. These data implicate σ receptors as the site of DTG antinociception, and more generally support the distinction between σ, opiate, and PCP/NMDA receptors.

Antinociception DTG σ Receptor Rimcazole Haloperidol MK-801 Naloxone Tail withdrawal Hypothermia Mice

BASED on their work with several classes of opioid compounds in the chronic spinal dog preparation, Martin and co-workers (41) proposed the existence of three types of opiate receptors designated μ, δ, and σ. The σ receptor was proposed to account for the dysphoric and psychotomimetic effects observed following the administration of certain opiate benzomorphans, such as *N*-allylnormetazocine (SKF 10,047), cyclazocine, and pentazocine. These σ opiates also produced potent pain inhibition in humans and animals (49,50).

However, the use of racemic enaniomers of the benzomorphans led to the erroneous characterization of the σ receptor as an opiate receptor subtype. It is now clear that the σ binding site displays opposite stereoselectivity (dextrorotatory < levorotatory) relative to classical opiate receptors [see (60)]. Naloxone and naltrexone, which are levorotatory, do not antagonize the in vitro and in vivo effects of ligands acting at the σ site (40,52,55,57,59). The observation that SKF 10,047, the prototypic σ ligand, and phencyclidine (PCP) produced similar behavioral responses in several species, and that σ opioids inhibit the binding of [^{3}H]PCP (7,8,29,35,43,54,55), led to the proposition that σ ligands exerted their effects by acting at a singular "σ/PCP" binding site (65). There is now considerable evidence that these two sites are distinct. The σ receptor displays high affinity for [^{3}H](+)-SKF 10,047 and is preferen-

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tially labelled by haloperidol. PCP binding sites have low affinity for [^{3}H](+)-SKF 10,047 and are located in the ion channel of the *N*-methyl-D-aspartate (NMDA) receptor (52).

Attempts to define a functional role for the σ receptor have previously been complicated by drugs that are nonselective for this site. Even (+)-SKF 10,047 has strong interactions with other brain binding sites. However, some functional data have recently become available via the use of ligands such as 1,3-di-*o*-tolylguanidine (DTG), which displays high selectivity and affinity for the σ receptor. Depending on the ligand used for displacement, DTG has an affinity for σ receptors several hundredfold greater than for PCP/NMDA receptors (15,56,61). DTG does not produce the behavioral effects typically observed following PCP administration, and DTG's physiologic effects are also not consistent with a PCP/NMDA receptor site of action (8,25,28,30,62) [see, however, (14)]. Therefore, DTG represents a suitable probe with which to evaluate the role of the σ receptor in nociceptive processes. In Experiment 1, the effect of the DTG on tail-withdrawal latencies in mice were examined. To confirm the role of σ receptors in DTG antinociception, animals in Experiment 2 were pretreated with the σ ligands haloperidol and rimcazole, which have been previously shown to antagonize effects attributable to DTG and other σ ligands (11,12,20,31,34,38,46,47). Furthermore, because racemic SKF 10,047, (+)-SKF 10,047, and other benzomorphan opiates used to characterize the σ receptor produce potent analgesia (1,16,23,35,50) consistent with an opiate or PCP/NMDA receptor site of action, mice were also pretreated with the opiate antagonist naloxone and the specific, noncompetitive PCP/NMDA antagonist MK-801 (45). Finally, because DTG has been reported to produce hypothermia in rats (5), a potential confounding factor in tests of thermal nociception such as tail withdrawal, the effect of DTG on body temperature, and the ability of rimcazole to antagonize this effect, was assessed in Experiment 3.

METHODS

Subjects

Adult male Swiss-Webster mice weighing 30–45 g were used in all experiments. Animals were housed five to a cage, and maintained on a 12 L : 12 D cycle (lights on at 08:00 h) in a temperature-controlled environment, with food and water available ad lib. Different mice were used in each separate experiment.

Drugs

The following drugs were used: 1,3-di-*o*-tolylguanidine (DTG; Aldrich Chemical Co., Milwaukee, WI), haloperidol (Research Biochemicals Inc., Natick, MA), MK-801 (dizocilpine; Research Biochemicals Inc.), naloxone (Sigma Chemical Co., St. Louis, MO), and rimcazole (Research Biochemicals Inc.). DTG was initially dissolved in a small volume of acetic acid, then enough sodium hydroxide and saline were added to neutralize (pH 7.0) the solution and obtain the desired final concentration. All other drugs were dissolved in 0.9% physiologic saline. Drugs were injected intraperitoneally in a volume of 10 ml/kg.

Nociceptive Testing

All testing occurred between 10:00 and 15:00 h. Nociceptive thresholds of all mice were assessed using the tail-withdrawal test. Briefly, mice were lightly restrained and the distal 4 cm of their tails immersed in a water bath kept at a constant temperature of 50 ± 0.2°C. The trial was terminated upon the reflexive removal of the tail from the water, and the latency recorded by use of a hand-held stopwatch. Latencies were composed of the mean of two determinations, separated by approximately 10 s. A cutoff latency of 10 s was employed to prevent the possibility of tissue damage.

Core Body Temperature

Separate groups of mice were used to assess core body temperature. A lubricated thermistor probe (YSI 400 Series; Yellow Springs, OH) connected to a digital thermometer (VWR Scientific Digital Thermometer 500; San Francisco, CA) was inserted 3 cm into the rectum. The probe was removed following stabilization of the temperature reading (±0.1°C).

Data Analysis

In Experiment 1, repeated measures ANOVAs were performed using raw latency data (i.e., preinjection latency vs. postinjection latency) to establish the existence of antinociception.

In Experiment 2, postinjection tail-withdrawal latencies were expressed as percentages of the maximum possible effect (%MPEs), as calculated by the formula:

$$\%\text{MPE} = \frac{(\text{postinjection latency} - \text{baseline latency})}{(\text{cutoff latency} - \text{baseline latency})} \times 100$$

The use of %MPEs takes into account differences in baseline nociceptive latencies so that these differences will not bias the quantification of antinociception. Group %MPE means were compared using a two-way ANOVA (agonist × antagonist) and then one-way ANOVAs for identification of simple main effects.

In Experiment 3, a repeated-measures ANOVA was performed on core body temperature data to establish the existence of hypothermia.

In all experiments, post hoc analyses were performed, where appropriate, using the Student-Newman-Keuls test. The criterion level of significance in all cases was $p < 0.05$.

Experiment 1: Effect of DTG on Nociception

Immediately following assessment of baseline withdrawal latency, mice received either saline (N = 21) or DTG (10, 15, or 20 mg/kg; N = 7 per dose), and nociceptive thresholds were assessed at 15, 30, 60, 90, and 120 min postinjection. Only one DTG dose was tested each day, with a minimum of 4 days between successive trials, along with a corresponding number of saline-treated controls. However, there were no day differences noted in either baseline or postinjection tail-withdrawal latencies of DTG- or saline-treated mice. Thus, all saline data were pooled (N = 21).

Experiment 2: Effect of σ, PCP/NMDA, and Opiate Antagonists on DTG Antinociception

Immediately following baseline determinations, subjects received an antagonist (N = 8–10 per group) or saline (N = 8) followed by DTG. Rimcazole (RIM; 10 and 25 mg/kg), naloxone (NAL; 5 mg/kg), and saline were administered immediately before DTG administration, and MK-801 (0.1 mg/kg) and haloperidol (HAL; 0.025 and 0.05 mg/kg) were administered 5 and 15 min, respectively, before DTG administration. Animals were then retested at 15 min following DTG (15

mg/kg) administration, corresponding to the dose and time found in Experiment 1 to yield peak antinociception. Separate groups of mice (N = 8 per group) serving as controls were pretreated with saline, MK-801, RIM (25 mg/kg), and HAL (0.025 and 0.05 mg/kg) according to the schedule described previously, but received saline instead of DTG and were retested 15 min later.

Experiment 3: Effect of DTG and Rimcazole on Core Body Temperature

Mice (N = 7) received one of the following drug pairs immediately after determination of basal core body temperature: saline plus saline, saline plus DTG (15 mg/kg), RIM plus saline (25 mg/kg), and RIM plus DTG (same doses). Fifteen minutes later, animals were retested for core body temperature.

RESULTS

Experiment 1

A one-factor-between (DTG dose), one-factor-within repeated-measures ANOVA revealed significant main effects of drug [$F(3, 285) = 9.41$], repeated measure [$F(5, 285) = 44.03$], as well as a significant drug × repeated-measure interaction [$F(15, 285) = 4.39$].

No significant differences between baseline tail-withdrawal latencies were noted [$F(3, 57) = 0.66$, NS]. Post hoc analyses revealed that the two higher DTG doses (15 and 20 mg/kg) produced significant increases in tail-withdrawal latency relative to saline at 15 and 30 min postinjection (Fig. 1). Peak levels of antinociception (tail-withdrawal latency increases of 2.5 s) were observed in both doses at 15 min. The low DTG dose (10 mg/kg) failed to produce significant antinociception at 15 min postinjection, but did so at 30 min.

It should be noted that the 20 mg/kg dose of DTG proved lethal in 10–20% of mice. There were no instances of lethality resulting from either of the lower doses (10 and 15 mg/kg). We did not observe ataxia or any other motoric effects as assessed by performance on an inclined plane (data not shown), even after the 20 mg/kg dose in surviving animals.

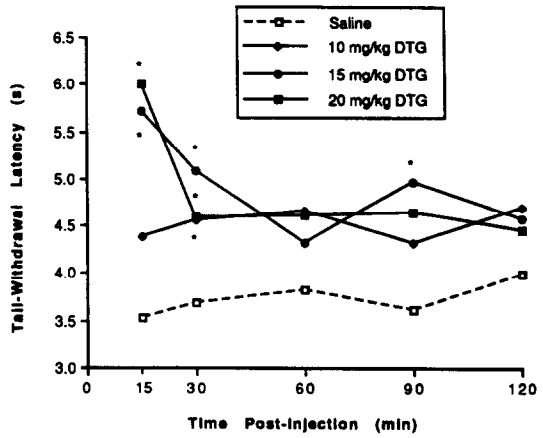

FIG. 1. Mean tail-withdrawal (50°C) latencies following 10, 15, and 20 mg/kg (IP) of DTG. Animals were tested immediately before, and 15, 30, 60, 90 and 120 min following drug administration. Saline values represent pooled scores from all experimental trials. Baselines (not shown) did not differ between groups. Error bars are omitted for purposes of clarity. *Significantly different from saline, $p < 0.05$.

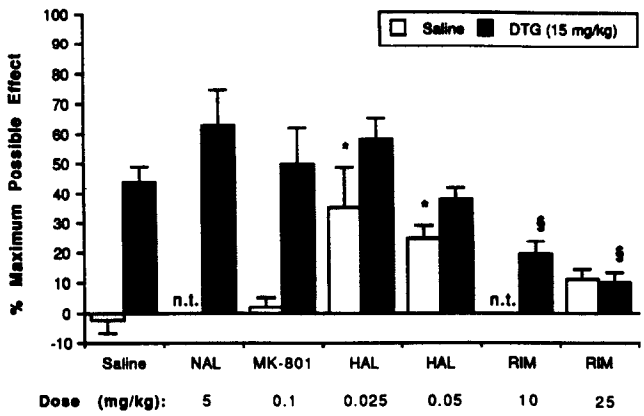

FIG. 2. The effect of saline, naloxone (NAL; 5 mg/kg), MK-801 (0.1 mg/kg), haloperidol (HAL; 0.025 and 0.05 mg/kg), and rimcazole (RIM; 10 mg/kg and 25 mg/kg) on nociceptive thresholds following either saline or 15 mg/kg DTG. Data are presented as percentages of the maximum possible effect. Bars represent means ± SEM. All groups receiving DTG displayed significant antinociception relative to the saline + saline group, except DTG + RIM (25 mg/kg). *Significantly different from baseline (saline + saline), $p < 0.05$. §Significantly different from DTG + saline, $p < 0.05$. n.t., Not tested.

Experiment 2

As in Experiment 1, there were no significant baseline differences between groups, and neither DTG nor any of the antagonists, alone or in combination, produced visible ataxia or motoric impairment. A two-way ANOVA revealed significant main effects of agonist [DTG vs. saline; $F(1, 100) = 37.53$] and antagonist [$F(6, 100) = 7.75$], as well as a significant agonist × antagonist interaction [$F(4, 100) = 7.07$]. A one-way ANOVA performed on mice receiving saline instead of an antagonist revealed a significant simple main effect of DTG [$F(1, 35) = 45.46$] (Fig. 2). The mean DTG antinociception in these animals was 43.6 %MPE, which was virtually identical to the level observed in Experiment 1. A one-way ANOVA performed on mice receiving saline instead of DTG revealed a significant simple main effect of antagonist [$F(4, 30) = 6.78$], indicating that at least one of the antagonists produced antinociception. Post hoc analysis demonstrated that only HAL, at both doses, was antinociceptive. A one-way ANOVA performed on mice receiving DTG as the agonist also revealed a significant simple main effect of antagonist [$F(6, 61) = 8.43$]. Post hoc analysis demonstrated a significant antagonism of DTG antinociception by both doses of RIM. No other antagonist significantly altered DTG antinociception.

Experiment 3

1,3-di-*o*-Tolylguanidine produced significant hypothermia [$F(1, 16) = 46.65$], but this effect was not altered by the prior administration of RIM [$F(1, 16) = 0.35$, NS]. RIM alone had no effect on core body temperature [$F(1, 8) = 0.35$, NS] (Table 1).

DISCUSSION

In the present study, DTG produced small but significant increases in nociceptive thresholds. Antinociception was not accompanied by gross motor impairment, a result similar to those reported for other species following systemic administration (5,8,62).

TABLE 1

EFFECTS OF DTG (15 mg/kg) AND RIMCAZOLE (25 mg/kg) ON CORE BODY TEMPERATURE

Group	N	BL	PI	PI − BL
Saline + saline	5	38.1 ± 0.1	38.0 ± 0.1	−0.1
Saline + DTG	5	38.3 ± 0.2	34.9 ± 0.6*	3.3†
Rimcazole + saline	5	38.2 ± 0.2	38.1 ± 0.2	−0.1
Rimcazole + DTG	5	38.2 ± 0.1	34.2 ± 0.9*	4.0†

BL, Baseline temperature; PI, Postinjection temperature. Values are expressed as mean ± SEM. *Significantly different from BL, $p < 0.05$. †Significantly different from saline + saline, $p < 0.05$.

The supposition that DTG's antinociceptive effect was mediated by activity at σ-binding sites is supported by the finding that 15 mg/kg DTG antinociception was significantly blocked by rimcazole (10 and 25 mg/kg) in a dose-dependent manner, but not by the opioid antagonist naltrexone (5 mg/kg) or the NMDA antagonist MK-801 (0.1 mg/kg). Rimcazole has good affinity and high selectivity for the σ receptor, with at least a 10–100 times higher affinity for σ-binding sites than at 16 other CNS receptor sites (18–20). Rimcazole has also been previously reported to antagonize physiologic and behavioral effects attributable to σ receptors and DTG (11,12,31). The failure of both naltrexone and MK-801 to block DTG antinociception in the present study is consistent with their lack of affinity for the σ-receptor site (17,40,52,55,57,59). However, the failure of MK-801 to block DTG antinociception does not necessarily preclude the converse: that DTG antinociception is affected through NMDA receptors. Because NMDA is pronociceptive (1,16), DTG may produce antinociception if it acts as an NMDA antagonist. In this case, the antinociceptive actions of DTG would not be blocked by MK-801, as was observed here, because both agents act in the same direction (i.e., NMDA antagonism). However, this possibility is not likely, as DTG antinociception was blocked by rimcazole, which has no affinity for the NMDA receptor, and NMDA antagonism had no antinociceptive effect in our behavioral paradigm (see MK-Sal group, Experiment 2). Furthermore, Delander and Wahl (16) reported that MK-801 and PCP, but not DTG, reduce NMDA-induced nociception following intrathecal administration. For these reasons, it does not appear that DTG produces antinociception by acting as an NMDA receptor antagonist. Thus, in addition to opioid receptors, PCP/NMDA receptors are also discounted as a putative site of DTG antinociception, consistent with the lack of even moderate affinity of DTG for either site (61).

In the study by Delander and Wahl (16), DTG was ineffective in reducing NMDA- and substance P-induced nociception following intrathecal administration. Although this observation seems to contrast with the present results, it may be attributable to the different routes of administration used in the two studies. Because all three drugs used in their study were administered intrathecally, any conclusions would be limited to the role of DTG and σ receptors in spinal mechanisms of antinociception. In the present study, DTG was administered systemically, allowing for the possibility that the drug interacted with any number of supraspinal loci that are involved in modulating nociception. Sites that have been reported to display high densities of DTG binding sites (42) and modulate pain reactivity include the dorsal raphe nucleus (2,3,37), locus coeruleus (26,27,33,44), habenula (6,13), and the paraventricular nucleus and medial preoptic area of the hypothalamus (10,63).

Haloperidol, which has high affinity for σ receptors (24, 58,60,64,66), potently displaces [^{3}H]DTG binding (22,53), and has putative antagonist effects at the σ site (17,38,52), also did not significantly alter DTG antinociception in the present study. However, haloperidol significantly increased nociceptive thresholds on its own. Therefore, any potential antagonistic effect of haloperidol on DTG antinociception may have been masked. A clear agonist-antagonist relationship for haloperidol at σ-binding sites has yet to be unequivocally determined (4,39), and thus a σ site of action in haloperidol antinociception is not inconceivable. Alternatively, haloperidol may have increased nociceptive thresholds by acting at receptor sites other than σ receptors. Indeed, haloperidol binds with high affinity to dopaminergic, α_1-adrenergic, serotonergic, and muscarinic receptors (9,48,51). Because these receptors have all been shown to have modulatory roles in nociceptive circuits [see (21)], it is difficult to ascribe the activity of haloperidol in the present study exclusively to a single site of action.

In agreement with previous studies using rats (5), we observed significant hypothermia in mice following DTG administration. This finding represents a potential confound of effects in nociceptive assays that use reactivity to thermal stimuli as an end point. However, it is unlikely that the increases in nociceptive thresholds observed presently in the tail-withdrawal test following DTG administration are a result of hypothermia, as only DTG antinociception, but not hypothermia, was blocked by rimcazole. This finding is consistent with the previously reported inability of rimcazole to block DTG hypothermia in the rat (5). Furthermore, DTG produces marked reductions in pain behavior following SC injection of formalin into the paw, a nonthermal test of nociception (36). Subtypes of the σ receptor have been described (32,60), and it is possible that DTG exerts its hypothermic effects at a σ-receptor subtype at which rimcazole has low affinity.

The modest magnitude of the DTG antinociception reported here may be more attributable to the method of nociceptive assessment used (tail withdrawal) than to the intrinsic analgesic activity of DTG. We have recently observed DTG antinociception of a considerably larger magnitude when assayed using the more sensitive formalin test (36). Although DTG is among the best of the current σ ligands, the in vivo pharmacology of DTG and its metabolites is not fully characterized at this time, and ascribing the present findings solely to a σ site of action must be made with caution. Furthermore, because the highest dose of DTG (20 mg/kg) produced 10–

20% mortality in mice, lower doses of the drug (10 and 15 mg/kg) could be toxic, although no motoric dysfunction or indications of malaise were apparent upon gross inspection. However, given that the psychotomimetic side effects of benzomorphan opiates are now thought not to be mediated at σ sites (49), a reevaluation of the clinical potential of σ ligands as antinociceptive agents seems warranted.

ACKNOWLEDGEMENTS

We wish to thank Mr. Herbert L. Washington and his staff for their excellent animal care, and Dr. M. F. Galazkiewicz for his helpful comments. This study was supported by NIH Grant NS07628 and an Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company. B.K. is supported by NIMH Postdoctoral Research Fellowship 5T32MH17140.

REFERENCES

1. Aanonsen, L. M.; Wilcox, G. L. Nociceptive action of excitatory amino acids in the mouse: Effects of spinally administered opioids, phencyclidine and σ agonists. J. Pharmacol. Exp. Ther. 243: 9–19; 1987.
2. Andersen, E.; Dafny, N. Dorsal raphe stimulation reduces responses of parafascicular neurons to noxious stimulation. Pain 15:323–331; 1983.
3. Andersen, E.; Dafny, N. An ascending serotonergic pain modulation pathway from the dorsal raphe nucleus to the parafascicularis nucleus of the thalamus. Brain Res. 269:57–67; 1983.
4. Beart, P. M.; O'Shea, R. D.; Manallack, P. J. Regulation of σ receptors: High- and low-affinity agonist states, GTP shifts, and upregulation by rimcazole and 1,3-di(2-tolyl)guanidine. J. Neurochem. 53:779–788; 1989.
5. Bejanian, M.; Pechnick, R. N.; Bova, M. P.; George, R. Effects of SC and intracerebroventricular administration of the *sigma* receptor ligand 1,3-di-*o*-tolylguanidine on body temperature in the rat: Interactions with BMY 14802 and rimcazole. J. Pharmacol. Exp. Ther. 258:88–93; 1991.
6. Benabid, A. L.; Mahieux, G. Electrical stimulation of the rat habenular complex induces a naloxone reversible analgesia. Soc. Neurosci. Abstr. 10:102; 1984.
7. Brady, K. T.; Balster, R. L.; May, E. L. Stereoisomers of *N*-allylnormetazocine: Phencyclidine-like behavioral effects in squirrel monkeys and rats. Science 215:178–180; 1982.
8. Brent, P. J. Similar behavioural effects of sigma agonists and PCP-like non-competitive NMDA antagonists in guinea-pigs. Psychopharmacology 105:421–427; 1991.
9. Burt, D. R.; Creese, I.; Snyder, S. H. Antischizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. Science 196:326–328; 1977.
10. Carstens, E.; MacKinnon, J. D.; Guinan, M. J. Inhibition of spinal dorsal horn neuronal responses to noxious skin heating by medial preoptic and septal stimulation in the cat. J. Neurophysiol. 48:981–991; 1982.
11. Ceci, A.; Smith, M.; French, E. D. Activation of the A10 mesolimbic system by the sigma-receptor agonist (+)SKF 10,047 can be blocked by rimcazole, a novel putative antipsychotic. Eur. J. Pharmacol. 154:53–57; 1988.
12. Coccini, T.; Costa, L. G.; Manzo, L.; Candura, S. M.; Iapadre, N.; Balestra, B.; Tonini, M. Two subtypes of enteric nonopioid sigma receptors in guinea-pig cholinergic motor neurons. Eur. J. Pharmacol. 198:105–108; 1991.
13. Cohen, S. R.; Melzack, R. Habenular stimulation produces analgesia in the formalin test. Neurosci. Lett. 70:165–169; 1986.
14. Contreras, P. C.; Contreras, M. L.; Compton, R. P.; O'Donohue, T. L. Biochemical and behavioral characterization of PCP and *sigma* receptors. In: Domino, E. F.; Kamenka, J.-M., eds. *Sigma* and phencyclidine-like compounds as molecular probes in biology. Ann Arbor, MI: NPP Books; 1988:327–334.
15. Contreras, P. C.; Contreras, M. L.; O'Donohue, T. L.; Lair, C. C. Biochemical and behavioral effects of sigma and PCP ligands. Synapse 2:240–243; 1988.
16. DeLander, G. E.; Wahl, J. J. In vivo characterization of phencyclidine/σ agonist-mediated inhibition of nociception. Eur. J. Pharmacol. 159:149–156; 1989.
17. Deutsch, S. I.; Weizman, A.; Goldman, M. E.; Moritresa, J. M. The sigma receptor: A novel site implicated in psychosis and antipsychotic drug efficacy. Clin. Neuropharmacol. 11:105–119; 1988.
18. Ferris, R.; Cooper, M.; Harfenist, M.; Maxwell, R. The pharmacology of a new nondopamine blocking antipsychotic, BW 234U. Clin. Neuropharmacol. 7(Suppl 1):146–147; 1984.
19. Ferris, R. M.; Harfenist, M.; McKenzie, G. M.; Cooper, B.; Soroko, F. E.; Maxwell, R. A. BW 234U,(cis-9-[3-(3,5-dimethyl-1-piperazinyl)propyl]carbazole dihydrochloride): A novel antipsychotic agent. J. Pharm. Pharmacol. 34:388–390; 1982.
20. Ferris, R. M.; Tang, F. L. M.; Chang, K.-J.; Russell, A. Evidence that the potential antipsychotic agent Rimcazole (BW 234U) is a specific, competitive antagonist of sigma sites in brain. Life Sci. 38:2329–2337; 1986.
21. Fields, H. L.; Heinricher, M. M.; Mason, P. Neurotransmitters in nociceptive modulatory circuits. Annu. Rev. Neurosci. 14:219–245; 1991.
22. Fischer, J. B.; Howie, K. J. B.; Dunn, J. A.; Ablordeppey, S. Y.; Glennon, R. A. Characterization of sigma subtype binding in guinea pig brain membranes. Soc. Neurosci. Abstr. 18:455; 1992.
23. Forrest, W. H. Jr.; Beer, E. G.; Bellville, J. W.; Ciliberti, B. J.; Miller, E. V.; Paddock, R. Analgesic and other effects of the *d*- and *l*-isomers of pentazocine. Clin. Pharmacol. Ther. 10: 468–476; 1969.
24. Gundlach, A. L.; Largent, B. L.; Snyder, S. H. Autoradiographic localization of σ-receptor binding sites in guinea pig and rat CNS with (+)-^{3}H-(3-hydroxyphenyl)-*N*-(1-propyl)-piperidine. J. Neurosci. 6:1757–1770; 1986.
25. Herling, S.; Coale Jr.; E. H.; Hein, D. W.; Winger, G.; Woods, J. H. Similarity of the discriminative stimulus effects of ketamine, cyclazocine, and dextrorphan in the pigeon. Psychopharmacology 73:286–291; 1981.
26. Hodge, C. J.; Apkarian, A. V.; Stevens, R. T.; Vogelsang, G. D.; Brown, O.; Frank, J. I. Dorsolateral pontine inhibition of dorsal horn cell responses to cutaneous stimulation: Lack of dependence on catecholaminergic system in cat. J. Neurophysiol. 50:1220–1235; 1983.
27. Hodge, C. J.; Apkarian, A. V.; Stevens, R. T.; Vogelsang, G. D.; Wisnicki, H. J. Locus coeruleus modulation of dorsal horn unit responses to cutaneous stimulation. Brain Res. 204:415–420; 1981.
28. Holtzman, S. G. Phencyclidine-like discriminative effects of σ opioids in the rat. J. Pharmacol. Exp. Ther. 214:614–619; 1980.
29. Holtzman, S. G. Phencyclidine-like dissociative stimulus properties of opioids in the squirrel monkey. Psychopharmacology 77: 295–300; 1982.
30. Holtzman, S. G. Opioid and phencyclidine-like discriminative effects of ditolylguanidine, a selective sigma ligand. J. Pharmacol. Exp. Ther. 248:1054–1062; 1989.
31. Hudzik, T. J. Sigma ligand-induced emesis in the pigeon. Pharmacol. Biochem. Behav. 41:215–217; 1992.
32. Itzhak, Y.; Stein, I. Sigma binding sites in the brain: An emerging concept for multiple sites and their relevance for psychiatric disorders. Life Sci. 47:1073–1081; 1990.
33. Jones, S. L.; Gebhart, G. F. Quantitative characterization of ceruleospinal inhibition of nociceptive transmission in the rat. J. Neurophysiol. 56:1397–1410; 1986.
34. Kamei, J.; Iwamoto, Y.; Kawashima, N.; Hitosugi, H.; Misawa, M.; Kasuya, Y. Involvement of haloperidol-sensitive σ-sites in antitussive effects. Eur. J. Pharmacol. 224:39–43; 1992.
35. Keats, A. S.; Telford, J. Subjective effects of nalorphine in hospitalized patients. J. Pharmacol. Exp. Ther. 119:370–377; 1957.

36. Kest, B.; Mogil, J. S.; Sternberg, W. F.; Pechnick, R.; Liebeskind, J. C. 1,3-Di-*o*-tolylguanidine (DTG) differentially affects acute and tonic formalin pain: Antagonism by rimcazole. Pharmacol. Biochem. Behav. (in press).
37. Klatt, D. S.; Guinan, M. J.; Culhane, E. S.; Carstens, E.; Watkins, L. R. The dorsal raphe nucleus: A reevaluation of its proposed role in opiate analgesia systems. Brain Res. 447:246–252; 1988.
38. Largent, B. L.; Wikstrom, H.; Snowman, A. M.; Snyder, S. H. Novel antipsychotic drugs share high affinities of σ receptors. Eur. J. Pharmacol. 155:345–347; 1988.
39. Manallack, D. T.; Beart, P. M. Up-regulation of rat cortical sigma receptors after subchronic administration of rimcazole and 1,3-di(2-tolyl)guanidine. Neuropharmacology 27:1201–1203; 1988.
40. Manallack, D. T.; Beart, P. M.; Gundlach, A. L. Psychotomimetic σ-opiates and PCP. Trends Pharmacol. Sci. 7:448–451; 1986.
41. Martin, W. R.; Eades, C. G.; Thompson, J. A.; Huppler, R. E.; Gilbert, P. E. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197:517–532; 1976.
42. McLean, S.; Weber, E. Autoradiographic visualization of haloperidol-sensitive sigma receptors in guinea-pig brain. Neuroscience 25:259–269; 1988.
43. Mendelsohn, L. G.; Kalra, V.; Johnson, B. G. Sigma opioid receptor: Characterization and co-identity with the phencyclidine receptor. J. Pharmacol. Exp. Ther. 233:597–602; 1985.
44. Mokha, S. S.; McMillan, J. A.; Iggo, A. Descending control of spinal nociceptive transmission: Actions produced on spinal multireceptive neurones from the nuclei locus coeruleus (LC) and raphe magnus (NRM). Exp. Brain Res. 58:213–226; 1985.
45. Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. The excitatory amino acid receptors: Their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu. Rev. Pharmacol. Toxicol. 29:365–402; 1989.
46. Monnet, F. P.; Debonnel, G.; De Montigny, C. *In vivo* electrophysiological evidence for a selective modulation of *N*-methyl-D-aspartate-induced neuronal activation in rat CA3 dorsal hippocampus by *sigma* ligands. J. Pharmacol. Exp. Ther. 261:123–130; 1992.
47. Monnet, F. P.; Debonnel, G.; Junien, J. L.; De Montigny, C. *N*-methyl-D-aspartate-induced neuronal activation is selectively modulated by sigma receptors. Eur. J. Pharmacol. 179:441–445; 1990.
48. Muller, P.; Seeman, P. Dopaminergic supersensitivity after neuroleptics: Time-course and specificity. Psychopharmacology 60: 1–11; 1978.
49. Mussachio, J. M. The psychotomimetic effects of opiates and the σ receptor. Neuropsychopharmacology 3:191–200; 1990.
50. Pasternak, G. W.; Carroll-Buatti, M.; Spiegel, K. The binding and analgesic properties of a *sigma* opiate, SKF 10,047. J. Pharmacol. Exp. Ther. 219:192–198; 1981.
51. Peroutka, S. J.; U'Prichard, D. C.; Greenberg, D. A.; Snyder, S. H. Neuroleptic drug interactions with norepinephrine a receptor binding sites in rat brain. Neuropharmacology 16:549–556; 1977.
52. Quirion, R.; Chicheportiche, R.; Contreras, P.; Johnson, K. M.; Lodge, D.; Tam, S. W.; Woods, J. H.; Zukin, S. R. Classification and nomenclature of phencyclidine and sigma receptor sites. Trends Neurosci. 10:444–446; 1987.
53. Rothman, R. B.; Mahboubi, A.; Reid, A. A.; de Costa, B. R.; Jacobson, A. E.; Rice, K. C. Interaction of calcium channel blockers with multiple sigma binding sites in guinea pig brain. In: Harris, L. (ed.), Proceedings of the Committee on Drug Dependence (NIDA Monograph Series). Bethesda, MD: USGPO; 1990.
54. Sanger, D. J.; Joly, D. Effects of NMDA receptor antagonists and sigma ligands on the acquisition of conditioned fear in mice. Psychopharmacology 104:27–34; 1991.
55. Shearman, G. T.; Herz, A. Nonopioid psychotomimetic-like discriminative stimulus properties of *N*-allylnometazocine (SKF 10047) in the rat. Eur. J. Pharmacol. 82:167–172; 1982.
56. Steinfels, G. F.; Tam, S. W.; Cook, L. Electrophysiological effects of selective σ-selective receptor agonists, antagonists, and the selective phencyclidine receptor agonist MK-801 on midbrain dopamine neurons. Neuropsychopharmacology 2:201–208; 1989.
57. Su, T.-P. Evidence for σ-opioid receptor: Binding of [^{3}H]-SKF-10047 to etorphine-inaccessible sites in guinea pig brain. J. Pharmacol. Exp. Ther. 223:284–290; 1982.
58. Tam, S. W.; Cook, L. Sigma opiates and certain antipsychotic drugs mutually inhibit (+)-[^{3}H]SKF 10047 and [^{3}H]haloperidol binding in guinea pig brain membranes. Proc. Natl. Acad. Sci. USA 81:5618–5621; 1984.
59. Vaupel, D. B. Failure of naltrexone to antagonize phencyclidine and the opioid SKF 10047 in the dog. Fed. Proc. 41:1333; 1982.
60. Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; De Costa, B.; Rice, K. C. Sigma receptors: Biology and function. Pharmacol. Rev. 42:355–401; 1990.
61. Weber, E.; Sonders, M.; Quarum, M.; McLean, S.; Pou, S.; Keana, J. F. W. 1,3-di(2-[5-^{3}H]tolyl-guanidine: A selective ligand that labels σ-type receptors for psychotomimetic opiates and antipsychotic drugs. Proc. Natl. Acad. Sci. USA 83:8784–8788; 1986.
62. Woods, J. H.; Koek, W.; Weber, E. Behavioural similarity of di-ortho-tolyl-guanidine (DTG), a selective ligand for a haloperidol-sensitive sigma binding site, to phencyclidine (PCP) in rats, pigeons and rhesus monkeys. Fed. Proc. 46:951; 1987.
63. Yirmiya, R.; Ben-Eliyahu, S.; Shavit, Y.; Marek, P.; Liebeskind, J. C. Stimulation of the hypothalamic paraventricular nucleus (PVN) produces analgesia not mediated by vasopressin or endogenous opioids. Brain Res. 537:169–174; 1990.
64. Zukin, R. S.; Zukin, S. R. Demonstration of [^{3}H]cyclazocine binding to multiple opiate receptor site. Mol. Pharmacol. 20:246–252; 1981.
65. Zukin, S. R.; Brady, K. T.; Silfer, B. L.; Balster, R. L. Behavioral and biochemical stereoselectivity of sigma opiate/PCP receptors. Brain Res. 294:174–177; 1984.
66. Zukin, S. R.; Sircar, R. Quantitative light microscopic visualization of sigma receptors with [^{3}H]TCP, [^{3}H](+)SKF 10047 and [^{3}H]PCP. Soc. Neurosci. Abstr. 11:584; 1985.