J. Pharm. Pharmacol. 1990, 42: 439–440
Communicated November 15, 1989

JO 1784, a potent and selective ligand for rat and mouse brain σ-sites

FRANÇOIS J. ROMAN, XAVIER PASCAUD, BRIGITTE MARTIN, DANIEL VAUCHÉ, JEAN-LOUIS JUNIEN, *Jouveinal Laboratories, B.P. 100, 94265 Fresnes, France*

Abstract—JO 1784 ((+)-cinnamyl-1-phenyl-1-*N*-methyl-*N*-cyclopropylene) is a potent ligand for (+)-[^{3}H]SKF 10,047 (2′-hydroxy-5,9-dimethyl-2-allyl-6,7-benzomorphan) binding sites in rat brain membrane preparations with an IC50 of 39±8 nM, which is comparable to that of haloperidol. The stereoisomer of JO 1784 is ten fold less potent. When administered to mice i.p. or p.o. JO 1784 displaced (+)-[^{3}H]SKF 10,047 (5 μCi i.v.) from its sites in the brain with ID50 values of 1·2 and 3·5 mg kg^{-1}, respectively. The high selectivity of JO 1784 for the σ-binding site was assessed by its lack of significant affinity for more than 20 other sites including those for phencyclidine.

The characteristics and functional role of σ- and phencyclidine sites in brain are currently under extensive investigation (Junien & Leonard 1989). Both sites have been identified in the brain (Largent et al 1986). σ-Sites are also found at the periphery (Wolfe et al 1987; Samovilova et al 1988; Roman et al 1988, 1989). It is now well established that the phencyclidine receptor is closely linked to the (*N*-methyl-D-aspartate NMDA) receptor complex (Anis et al 1983) whereas the role of the σ-site remains obscure partly because specific and useful ligands are missing. Recently 1,3-di-*o*-tolylguanidine (DTG) has been proposed to be a highly selective ligand for this site (Weber et al 1986). The purpose of this study was to describe the binding characteristics of JO 1784 ((+)-cinnamyl-1-phenyl-1-*N*-methyl-*N*-cyclopropylene), a novel molecule with high affinity for σ-binding sites.

Materials and methods

Animals. Male Sprague-Dawley rats (Iffa-Credo, France), 200–225 g, were used for binding assays in-vitro. Male Swiss mice (Iffa Credo, France), 20–22 g, were used for binding studies in-vivo. The animals were maintained in a room with controlled temperature (22–24°C) in a fixed light (07·00–19·00 h) and dark schedule, with food and water freely available.

σ-Site and phencyclidine binding assays. Rats were decapitated and the brains were removed rapidly and homogenized in 30 volumes of 50 mM Tris-HCl buffer pH 7·4 at 4°C with a Kinematica Polytron (Kriens-Lu, Switzerland) setting 5, 2 × 15 s. The homogenate was centrifuged at 49000 *g* for 15 min and the pellet was resuspended in 30 volumes of the same buffer. After incubation at 37°C for 45 min under shaking, the homogenate was centrifuged again. The pellet was washed again and the resulting homogenate was used for binding assays.

σ-Receptor sites were characterized using (+)-[^{3}H]SKF 10,047 (2′-hydroxy-5,9-dimethyl-2-allyl-6,7,-benzomorphan, 40 Ci mmol^{-1}, NEN, France) according to the method of Largent et al (1986). Phencyclidine receptor sites were characterized using [^{3}H]1-(1-(2-thienyl)cyclohexyl) piperidine ([^{3}H]TCP, 46 Ci mmol^{-1}, CEA, France) according to Vignon et al (1986).

Briefly, the homogenate was centrifuged at 49000 *g* for 10 min at 4°C and the pellet was resuspended in the same volume of 5 mM Tris-HCl buffer pH 7·4. Fractions (150 μL) were incubated at 25°C for 1 h in a total volume of 250 μL containing (+)-[^{3}H]-SKF 10,047 or [^{3}H]TCP at a final concentration of 3 nM. Incubation was terminated by filtration under reduced pressure through Whatman GF/B glass-fibre circles that had been soaked for at least 4 h in a solution of 5 mM Tris-HCl containing 0·1% polyethyleneimine. Filters were washed twice with 5 mL of cold Tris buffer and placed in minivials with 5 mL Scintillator 299 (Packard). Radioactivity was determined by scintillation counting in a Packard 4000 spectrometer. Non-specific binding was defined as the difference in radioactivity obtained in the absence and in the presence of 1 μM haloperidol for σ-receptors or 10 μM TCP for phencyclidine receptors. IC50 values from competitive inhibition studies were determined by a non-linear curve fitting computer program (LIGAND, McPherson 1985).

Correspondence to: F. J. Roman, Jouveinal Laboratoires, B.P. 100, 94265 Fresnes, France.

Other in-vitro binding assays. Using the classical binding assays routinely run in our laboratory with rat brain membranes, up to 23 binding receptor sites were tested including μ, δ, κ opioid, muscarinic M_1 and M_2, α_1-, α_2-, β_1- and β_2-adrenergic, dopaminergic D_1 and D_2, 5-hydroxytryptamine 5-HT_1, 5-HT_{1A}, 5-HT_{1B} and $5HT_2$, histaminergic H_1 and H_2, gabaergic$_A$, benzodiazepine central, glutamate$_{A1}$ and $_{A2}$, dopamine, noradrenaline, and 5-HT reuptake, calcium channels and adenosine-1 binding sites.

In-vivo σ-site binding assay. (+)-[^{3}H]SKF 10,047 binding in-vivo was assayed according to Ferris et al (1986). Mice were treated i.p. or p.o. with either 0·9% NaCl or test drug at several times before the i.v. tail vein injection of 5 μCi of (+)[^{3}H]SKF 10,047. Thirty min later, the mice were decapitated and the brains were rapidly removed, and homogenized in 40 volumes (w/v) of 50 mM Tris-HCl buffer pH 7·4. Four samples of 0·5 mL of this homogenate were filtered through Whatman GF/B filters presoaked overnight with 0·1% polyethyleneimine, washed twice with 5 mL ice-cold buffer, placed in minivials and counted as previously described. The non-specific binding was determined using brain homogenates obtained from mice injected with 2 mg kg^{-1} i.p. of haloperidol.

Results and discussion

Table 1 shows the IC50 values of JO 1784 and various reference drugs for σ- and phencyclidine-sites. JO 1784 potently inhibited (+)-[^{3}H]SKF 10,047 binding to σ- sites with an IC50 of 39±8 nM. Its affinity was similar to that of haloperidol (24±6 nM) which has been found to be one of the most active compounds for this site (Largent et al 1986). JO 1784 was more potent than

Table 1. Inhibition of (+)-[^{3}H]SKF 10,047 and [^{3}H]TCP binding to rat brain membranes by JO 1784, its isomer JO 1783 and reference drugs. Data are mean IC50 (nM)±s.e.m.; numbers in parentheses represent the number of experiments.

Compound	(+)-[^{3}H]SKF10,047		[^{3}H]TCP	
JO 1784	39·0±8·0	(5)	18990±4150	(3)
JO 1783	391±46	(4)	15160±7576	(3)
Haloperidol	24·6±6·7	(6)	85540±14562	(3)
(+)-SKF10,047	98·0±16	(4)	343±58	(4)
Pentazocine	38±5	(3)	2879±201	(3)
DTG	253±57	(3)	9024±1635	(3)
Phencyclidine	601±370	(4)	61·0±13·5	(3)
Rimcazole	2649±634	(4)	38337±12073	(3)

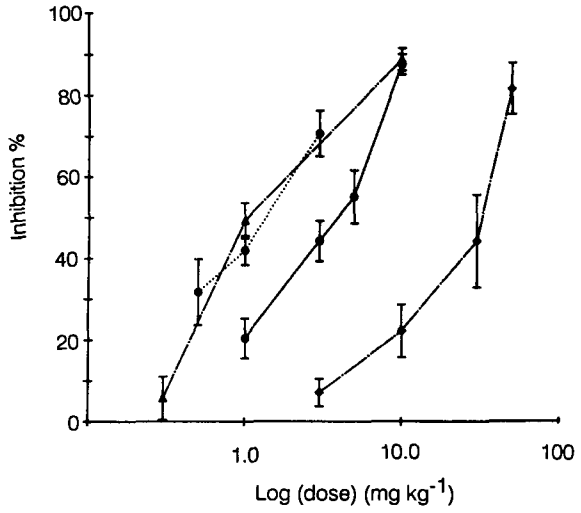

FIG. 1. Displacement of in-vivo particulate-bound (+)-[^{3}H]SKF 10,047 in mouse brain by JO 1784 administered i.p. (○) or p.o. (●), JO 1783 p.o. (◆) and DTG i.p. (▲)-Mice were treated with each drug 30 min prior to an i.v. injection of (+)-[^{3}H]SKF 10,047 (5 μCi/mouse). Data symbols represent the averages of four values from four to five mice. Bars around each symbol represent the s.e.m. values.

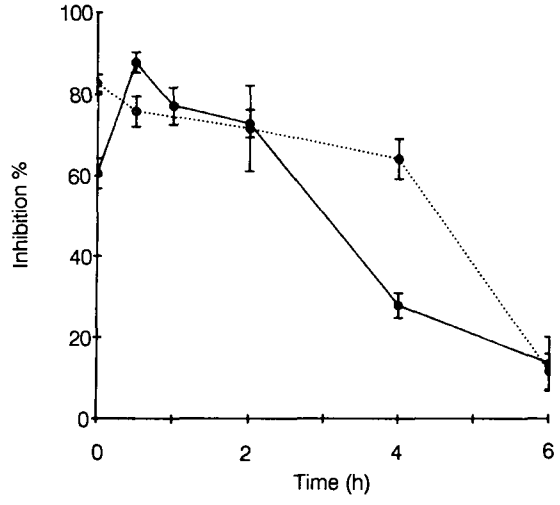

FIG. 2. Time course of the effect of JO 1784 on in-vivo particulate-bound (+)-[^{3}H]SKF 10,047 in whole mouse brain. Mice were injected with JO 1784 3 mg kg^{-1} i.p. (○) or 10 mg kg^{-1} p.o. (●) at several times before an i.v. injection of (+)-[^{3}H]SKF 10,047 (5 μCi/mouse). Data symbols represent the averages of four values from four to five mice. Bars around each symbol represent the s.e.m. values.

DTG (253 ± 57 nM). Rimcazole, which also may be a selective ligand for the σ-receptor (Ferris et al 1986) has lower affinity. JO 1783, the stereoisomer of JO 1784 was ten times less potent than JO 1784, indicating a certain degree of stereospecificity of the compound for the σ-site. In addition, JO 1784 displayed 500 fold less affinity for the phencyclidine receptor labelled by [^{3}H]TCP. (+)-SKF 10,047, as reported by others (e.g. Largent et al 1986), displaced [^{3}H]TCP with moderate potency (302 ± 58 nM).JO 1784, at 10^{-5} M, had no significant affinity for 23 other receptors tested (listed in methods section). In-vivo, JO 1784 also inhibited the specific binding of (+)-[^{3}H]SKF 10,047 to the brain σ- sites of mice. The ID50 values were 3·5 and 1·2 mg kg^{-1} for the oral and i.p. route, respectively (Fig. 1). The affinity of DTG was similar to that of JO 1784 under the same conditions. JO 1783 was ten fold less potent than its (+)-stereoisomer (ID50 = 36·5 mg kg^{-1}). JO 1784 induced no toxic effects before 600 mg kg^{-1} orally (LD50 > 1000 mg kg^{-1}) while DTG induced convulsions at doses as low as 20 mg kg^{-1}, limiting its use for in-vivo pharmacology. The marked effect of JO 1784 was found to be long-lasting; 4 h after p.o. or i.p. administration, (+)-[^{3}H]SKF 10,047 binding was inhibited by at least 30 or 60%, respectively (Fig. 2).

These results demonstrate that JO 1784 is a potent and specific ligand for σ- sites with virtually no affinity for phencyclidine sites in rodent brain. With respect to σ-binding, JO 1784 has higher affinity than DTG in-vitro and is equipotent in-vivo. In contrast to DTG, JO 1784 is not convulsant at low or moderate doses and, thus, may be considered as a valuable agent for evaluating the functional role of σ-receptors.

References

Anis, N. A., Berry, S. C., Burton, N. R., Lodge, D. (1983) The dissociative anesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurons by N-methylaspartate. Br. J. Pharmacol. 79: 515–575

Ferris, R. M., Tang, F. L. M., Chang, K. J., Russel, A. (1986) Evidence that the potential antipsychotic agent rimcazole (BW 234 U) is a specific, competitive antagonist of sigma sites in brain. Life Sci. 38: 2329–2337

Junien, J. L., Leonard, B. E. (1989). Drugs acting on sigma and phencyclidine receptors: a review of their nature, function, and possible therapeutic importance. Clin. Neuropharmacol. 12: 353–374

Largent, B. L., Gundlach, A. L., Snyder, S. H. (1986) Pharmacological and autoradiographic discrimination of sigma and phencyclidine receptor binding sites in brain with (+)-3H SKF 10,047, (+)-3H-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine and 3H-1-(2-thienyl) cyclohexyl piperidine. J. Pharmacol. Exp. Ther. 238: 739–749

McPherson, G. A. (1985) Analysis of radioligand binding experiments: a collection of computer programs for the IBM PC. J. Pharmacol. Methods 14: 213–228

Roman, F., Pascaud, X., Vauché, D., Junien, J. L. (1988) Evidence for a non-opioid sigma binding site in the guinea-pig myenteric plexus. Life Sci. 42: 2217–2222

Roman, F., Pascaud, X., Chomette, G., Bueno, L., Junien, J. L. (1989) Autoradiographic localization of sigma opioid receptors in the gastrointestinal tract of the guinea pig. Gastroenterology 97: 76–82

Samovilova, N. N., Nagornaya, L. V., Vinogradov, V. A. (1988) (+)[^{3}H]SKF10,047 binding sites in rat liver. Eur. J. Pharmacol. 147: 259–264

Vignon, J., Privat, A., Chaudieu, I., Thierry, A., Kamenka, J. M., Chicheportiche, A. (1986) [^{3}H]Thienyl-phencyclidine ([^{3}H]TCP) binds to two different sites in rat brain. Localization by autoradiographic and biochemical techniques. Brain Res. 378: 133–141

Weber, E. M., Sonders, M., Quarum, M., McLean, S., Pou, S., Keana, J. F. W. (1986) 1,3-Di(2-(5-3H-tolyl)guanidine: a selective ligand that labels sigma type receptors for psychotomimetic opiates and antipsychotic drugs. Proc. Natl. Acad. Sci. 83: 8784–8788

Wolfe, S. A., Kulsakdinum, C., De Souza, E. B. (1987) Sigma receptors in human peripheral blood leukocytes (HPBL) and rat spleen: identification, characterization and autoradiographic localization. Soc. Neurosci, 13: 1437