The Journal of Neuroscience, January 1995, 15(1): 731–736

Sigma Ligands Indirectly Modulate the NMDA Receptor–Ion Channel Complex on Intact Neuronal Cells via σ1 Site

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To investigate the modulatory effects of σ ligands on the *N*-methyl-D-aspartate (NMDA) receptor-ion channel complex *in vivo*, we examined the intact cell binding of ^{3}H-*N*-[1-(2-thienyl)cyclohexyl]piperidine (^{3}H-TCP) to cultured neuronal cells prepared from fetal rat telencephalon. The ^{3}H-TCP binding was saturable, reversible, and inhibited by a selective NMDA receptor antagonist, D-amino-5-phosphonovaleric acid. Millimolar Mg^{2+} inhibited ^{3}H-TCP binding both in the absence and presence of L-glutamate. 5-Methyl-10,11-dihydro-5H-dibenzo [a,d]cyclohepten-5,10-imine maleate (MK801) inhibited ^{3}H-TCP intact cell binding in a competitive manner, while haloperidol inhibited it in a noncompetitive manner. The effect of the test drugs to inhibit ^{3}H-TCP intact cell binding was in the order of dextromethorphan, haloperidol > (±)MK 801 > (+)pentazocine > (−)pentazocine > DTG > PCP > (+)-*N*-allylnormetazocine [(+)SKF 10047] > (+)3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [(+)3-PPP] > (−)SKF 10047 > (−)3-PPP. The IC_{50} values of the six σ ligands for ^{3}H-TCP binding were closely correlated with the K_i values of the corresponding drugs for DTG site 1 in the guinea pig brain reported by Rothman et al. (1991). These findings suggest that the σ ligand indirectly modulates the NMDA receptor ion channel complex, presumably through σ1 sites *in vivo* as well as *in vitro*.

[Key words: antipsychotic, ion channel, intact cell binding, NMDA receptor, σ1, primary culture]

The therapeutic efficacy of antipsychotic drugs is thought to be related to their potency in inhibiting dopaminergic transmission along mesolimbic and mesocortical pathways. However, the effective drugs are known to induce extrapyramidal side effects that may be due to the blockade of the D2 subclass of postsynaptic dopamine (DA) receptors in the striatum. Thus, an ideal antipsychotic drug would principally influence the cognitive and affective symptomatology of psychosis, and lack extrapyramidal side effects.

Received May 5, 1994; revised June 30, 1994; accepted July 14, 1994.

We express our thanks to Dr. T. Hama (Mitsubishi Kasei Institute of Life Sciences) for technical advice on preparing the primary cultured neuronal cells and to Mr. M. Okuwa (Sionogi Pharmaceutical Company Ltd.) for editorial assistance. This study was supported by a Grant-in-Aid for Scientific Research (No. 04770127) from The Ministry of Education, Science and Culture of Japan.

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The search for an understanding of psychotimimetic opiates led to identification and characterization of σ binding sites. Additionally, the evidence showing that some antipsychotic drugs (e.g., haloperidol and remoxipride) bind to σ binding sites with high affinity (Su, 1982; Tam and Cook, 1984; Largent et al., 1986; Ferris et al., 1991) has opened new strategies for developing a new class of antipsychotic drug, devoid of actions at DA receptors, that possesses selective high affinity for σ binding sites.

N-allylnormetazocine (SKF-10,047), a prototypic benzomorphan, and PCP were originally thought to elicit psychotimimetic effects via a common receptor, designated the σ/PCP site. However, subsequent studies demonstrated that the σ binding sites are distinct from PCP binding sites (PCP receptor) located within the cation channel of the *N*-methyl-D-aspartate (NMDA) receptor ion channel complex (Quirion et al., 1987; Deutsch et al., 1988; Church and Lodge, 1990; Sanger et al., 1991). Based on data obtained from biochemical and radioligand-binding experiments, subtypes of σ binding sites have been classified into at least two types designated σ1 and σ2, though the physiological roles for these two subtypes remains to be clarified (Walker et al., 1990).

Selective σ ligands such as (+)3-[3-hydroxyphenyl]-*N*-(1-propyl)-piperidine [(+)3-PPP] and 1,3-di-(2-tolyl)guanidine (DTG) have been reported to modulate the NMDA response either *in vitro* or *in vivo* experiments. Using intracellular and extracellular recording techniques, Malouf et al. (1988) have shown that although PCP blocks the pyramidal cell response to NMDA at behaviorally relevant concentrations (1–10 μM), (+)3-PPP (1 μM to 1 mM) enhances excitations in CA1 hippocampal pyramidal cells mediated by NMDA. DTG (1–10 μM) reverses or inhibits the enhancement of the NMDA response by (+)3-PPP, while application of 100 μM of DTG alone to the bath has no effect on the NMDA response. On the other hand, Monnet et al. (1992) have reported that DTG increases the neuronal firing activity induced by microiontophoretic application of NMDA to the rat CA3 dorsal hippocampus when injected intravenously at low doses ranging from 0.5 to 3 μg/kg. At low doses that do not by themselves affect the NMDA response, haloperidol, (+)3-PPP, and a-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperidinebutanol (BMY-14802) reverses DTG-induced potentiations of the NMDA response. The reason for the discrepancy between the above-cited observations is not clear, but may be attributable to differences in the experimental procedures used, i.e., the *in vitro* and *in vivo* experiments. Using cultured neuronal cells prepared from the fetal rat telencephalon, thus, we ex-

amined the modulation of the NMDA response by σ ligands to investigate the interaction of σ ligands with the NMDA receptor-ion channel complex *in vivo*.

Materials and Methods

Materials. ^{3}H-*N*-[1-(2-thienyl)cyclohexyl]piperidine (^{3}H-TCP, 1839 GBq/mmol) was purchased from New England Nuclear (Boston, MA). PCP and 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclo-hepten-5,10-imine maleate (MK-801) were donated from Shionogi Pharmaceutical Co. Ltd. (Osaka, Japan). Pentazocine was donated from Taisho Pharmaceutical Co. Ltd. (Saitama, Japan). Tetrodotoxin (TTX) and dextromethorphan were purchased from Sigma Chemical Co. (St.Louis, MO). NMDA and D-amino-5-phosphonovaleric acid (APV) were purchased from Cambridge Research Biochemicals, Ltd. (Harston, Cambridge, England). DTG, (+)3-PPP and SKF 10,047 were obtained from Research Biochemicals Inc. (Natick, MA). Other chemicals were purchased from commercial sources.

Cell culture. Primary neuronal cell cultures were prepared by mechanoenzymatic dissociation of the fetal rat telencephalon, according to a modification of the method described by Yuzaki et al. (1990). In brief, the rat embryos of gestational days 18 or 19 were removed under ether anesthesia, and then the telencephalon, including the cortex, hippocampus, and striatum, was dissected out and freed from the meninges. Following treatment with trypsin (0.25%, Difco) in Ca^{2+}/Mg^{2+}-free phosphate buffered saline (CMF-PBS) containing 0.02% DNase I (Sigma) for 15–20 min at 37°C on the shaker, the enzyme solution was removed by aspiration. To inactivate the residual enzyme, a mixture of equal parts of Dulbecco's modified Eagle's medium (DMEM) and the heat-inactivated horse serum was added to the cells. The cells were mechanically dispersed by repetitive pipetting and filtered through a nylon mesh to remove undissociated cells. The cells were rinsed three times with culture medium and plated on a poly-L-lysine (Sigma)-coated well (0.32 cm^2) at a final density of 0.5–1.5 × 10^6 cells per ml. The culture was maintained for 7 d with defined culture medium: DMEM supplemented with 1 mg/ml bovine serum albumin, 10 μg/ml insulin, 1 nM 3,3',5-triiodo-L-thyronine, 0.1 mg/ml human transferrin, 1 μg/ml aprotinin, 100 μM putrescine, 1 mM sodium pyruvate, 10 nM progesterone, 30 nM selenium, 0.1 mg/ml streptomycin sulfate, 50 units/ml penicillin G potassium salt (Meiji Seika Kaisha, Ltd., Tokyo, Japan) in a humidified atmosphere of 5% CO_2 in air at 37°C.

Representative cultures were stained to determine the relative proportions of glia. Glia were labeled by staining with immunohistochemical staining kit for glial fibrillary acidic protein (Biomeda Corp., Foster City, CA).

^{3}H-TCP intact cell binding. After removal of the media, the cells were washed three times with CMF-PBS. After the addition of 75 μl of Locke medium of modified composition (LMC) [(in mM) NaCl, 154; KCl, 5.6; $CaCl_2$, 1.8; sucrose, 1; $NaHCO_3$, 6; and glucose, 10; TTX 0.0005; buffered to pH 7.2 with 2 mM HEPES], the cells were preincubated with or without the drugs dissolved in 0.32 M sucrose solution except for NMDA and Mg^{2+} for 30 min in a humidified atmosphere of 5% CO_2 in air at 37°C.

The binding was started by the addition of 25 μl of 20 nM of ^{3}H-TCP with or without NMDA and Mg^{2+} in LMC in an atmosphere consisting of 95% O_2:5% CO_2 at 25°C (total volume; 100 μl/well), and was terminated by rapid aspiration of the media, followed by washing four times with ice-cold CMF-PBS in 10 sec. The cells were solubilized by the addition of 150 μl of 0.1 M NaOH. The resulting aliquot (100 μl) was neutralized with 0.1 M HCl, and the radioactivity was measured by liquid scintillation spectrophotometry (Beckman LS 9800) (Beckman, Fullerton, CA) in a 3 ml of Aquasol (New England Nuclear, Boston, MA) at a counting efficiency of 45–50%. Nonspecific binding was determined by the addition of PCP (1 mM). Protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad, Richmond, VA).

Binding of ^{3}H-TCP to the membrane preparation. Binding of ^{3}H-TCP to the membrane preparation was also examined. Extensively washed synaptic membranes were prepared from the cerebral cortex of male Wistar rats each weighing 150–250 gm, according to the method described in detail elsewhere (Hori et al., 1990). In brief, the cortical tissue was homogenized in ice-cold 0.32 M sucrose and centrifuged at 900 × *g* for 10 min at 4°C. The supernatant was centrifuged at 20,000 × *g* for 20 min at 4°C, and the crude synaptosomal membrane obtained was suspended in 50 mM Tris-HCl buffer containing 10 mM EDTA (pH 7.6), preincubation for 10 min at 37°C, and centrifuged at 20,000 × *g* for 20 min at 4°C. The resulting pellet was resuspended in the same buffer and quickly frozen, followed by thawing and centrifugation at 20,000 × *g* for 20 min at 4°C. The resulting pellet was resuspended in 5 mM Tris-HCl buffer (pH 7.6), frozen, and stored at −80°C for at least 24 hr prior to the binding assay. On the day of the experiment, the membrane was thawed at room temperature and washed twice with the assay buffer (5 mM Tris-HEPES buffer, pH 7.6). Finally, the resulting pellet was resuspended in the assay buffer.

The membrane homogenates in the assay buffer (8–10 μg protein in a final volume of 200 μl) were incubated with 5 nM ^{3}H-TCP for 60 min at 25°C. The incubation was terminated by rapid vacuum filtration through Whatman GF/C filters, using an Inotech Cell Harvester (Wohlen, Switzerland), followed by washing 10 times with ice-cold 5 mM Tris-HEPES buffer (pH 7.6). To reduce adsorption to them, the glass filters were presoaked for 15–20 min in 0.05% polyethylenimine before filtration. The radioactivity bound to the filters was measured by liquid scintillation spectrometry carried out in 3 ml of Aquasol, as mentioned above. Protein concentrations were determined as described by Lowry et al. (1951) using bovine serum albumin as the standard.

Statistics. Comparisons between groups were made using Student's *t* test (two tailed). Equilibrium saturation and drug competition binding data were analyzed with the iterative curve-fitting computer programs EBDA (McPherson, 1983) and LIGAND (Munson and Rodbard, 1980).

Results

More than 95% of the cultured cells used were non-GFAP immunoreactive when the positive control of cultured glial cells was stained well. Thus, the cultured neuronal cells used seemed to be suitable for studying ^{3}H-TCP binding to intact cell binding.

As shown in Figure 1*A*, specific ^{3}H-TCP intact cell binding was time dependent, an equilibrium being reached starting at 7 min. Thus, an incubation time of 10 min was used in subsequent experiments. The total binding was approximately 300–1000 d.p.m. per well. Nonspecific binding represents only 3.0–5.4% of the total binding at 5 nM ^{3}H-TCP. The dissociation reaction was initiated by the addition of excess of either PCP (100 μM) or haloperidol (100 μM). PCP more quickly and markedly stimulated dissociation of ^{3}H-TCP from the intact cells than haloperidol (Fig. 1*B*).

In a previous study, we showed that L-glutamate stimulated ^{3}H-TCP binding to extensively washed rat cortical membranes in a concentration-dependent manner, with a maximal effect, occurring in the concentration range of 10–100 μM, approximately 700% of the basal binding (Hori et al., 1990). On the other hand, Mg^{2+} had a biphasic effect on ^{3}H-TCP binding, i.e., a stimulatory effect at low concentrations and an inhibitory effect at high concentrations (Hori et al., 1991). Thus, we examined the effects of both L-glutamate and Mg^{2+} on the ^{3}H-TCP binding to the intact neuronal cells. In contrast to the ^{3}H-TCP binding to the membrane preparation, L-glutamate, at a concentration of 0.5 mM, had no stimulatory effect on the ^{3}H-TCP intact cell binding compared to the control binding (Fig. 2*B*). Similarly, Mg^{2+} at low concentrations did not stimulate ^{3}H-TCP intact cell binding in either the presence or absence of 0.5 mM L-glutamate, whereas Mg^{2+} concentrations higher than 1 mM inhibited ^{3}H-TCP binding (Fig. 2*A,B*). It should be noted that in both the absence or the presence of 0.5 mM L-glutamate, a selective NMDA antagonist, APV (1 mM), inhibited ^{3}H-TCP binding to the intact cells (Fig. 2*B*). Scatchard analysis of the saturation data indicated a single class of binding sites for ^{3}H-TCP in the intact cells with a $K_d = 25.8 \pm 3.4$ nM and a $B_{max} = 6.62 \pm 0.35$ pmol/mg protein ($N = 7$) (Fig. 3). The addition of 3 μM haloperidol markedly reduced the density of the NMDA receptor-ion channel complex on the intact cells without affecting the affinity of ^{3}H-TCP for its binding sites. By contrast, the addition

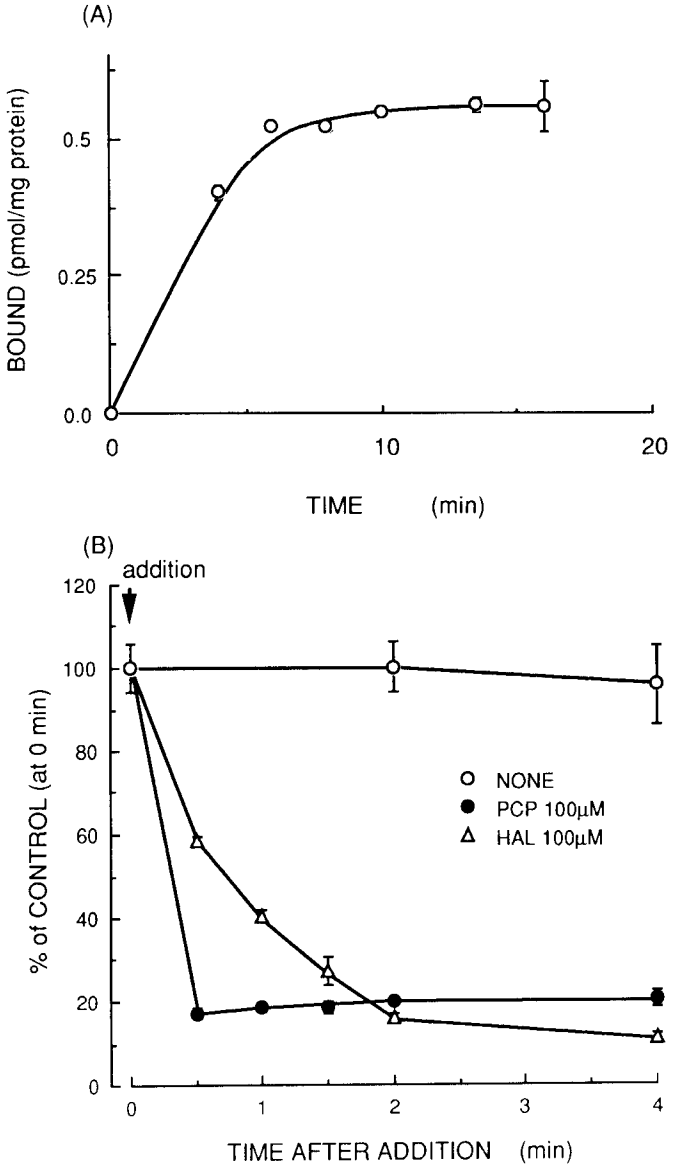

Figure 1. Association (*A*) and dissociation (*B*) curves for the binding of ^{3}H-TCP to the primary neuronal cultured cells at 25°C. As described in Materials and Methods, cells were incubated with 5 nM ^{3}H-TCP until reaching an equilibrium, followed further incubation with 100 μM of PCP or haloperidol, and then quickly washed and harvested. Each value is the average of triplicate determinations from a representative experiment that was repeated three times with similar results.

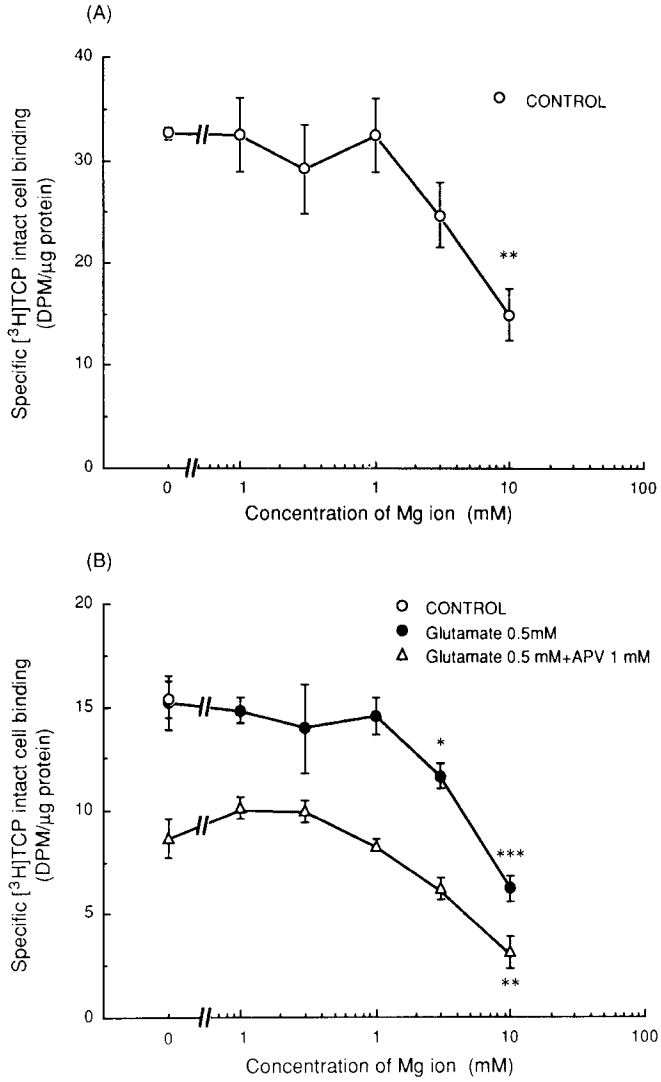

Figure 2. Effect of Mg^{2+} (*A*), glutamate, and an NMDA receptor antagonist APV (*B*) on ^{3}H-TCP intact cell binding. ^{3}H-TCP intact cell binding assays were conducted with increasing concentrations of magnesium ion. Each value is the average of triplicate (*A*) and quadruplicate (*B*) determinations from a representative experiment that was repeated two times with similar results.

of 3 μM (±)MK 801 reduced the affinity of ^{3}H-TCP binding sites without affecting receptor density.

Both antagonists of the NMDA receptor-ion channel complex and σ ligands were examined for their inhibition of ^{3}H-TCP binding to the intact cells. All the drugs tested had inhibitory effects, although MK-801 and (+)pentazocine at low concentrations (100 nM) stimulated ^{3}H-TCP binding (Fig. 4*A,B*), and the slope factors calculated using the LIGAND program (Hill coefficients) were in the range of 0.830–1.386. Of all the drugs tested, dextromethorphan (IC_{50} = 1.760 ± 0.41 μM) caused the greatest inhibition of ^{3}H-TCP intact cell binding. Haloperidol (IC_{50} = 1.863 ± 0.391 μM) inhibited ^{3}H-TCP binding to a similar extent as well. The order of inhibition was dextromethorphan, haloperidol > (±)MK-801 > (+)pentazocine > (−)pentazocine > DTG > PCP > (+)SKF 10047 > (+)3-PPP > (−)SKF 10047 > (−)3PPP (Table 1). The (+) isomers of the σ drugs were more potent than their (−) isomers. Representative NMDA antagonists and σ ligands were also examined for their ability to inhibit ^{3}H-TCP binding to an extensively washed rat cortical membrane preparation. Inhibition of ^{3}H-TCP binding to the membrane preparation by (±)MK-801 and PCP was 253 and 500 times higher than their inhibitory effects on ^{3}H-TCP intact cell binding, respectively. DTG and (+)SKF 10047 were less effective in inhibiting ^{3}H-TCP intact cell binding. By contrast, the inhibitory effect of haloperidol on ^{3}H-TCP intact cell binding was 10 times greater than that on ^{3}H-TCP binding to rat cortical membranes.

The IC_{50} values of six σ ligands obtained from the inhibition curves in the ^{3}H-TCP intact cell binding study were compared with the inhibitory dissociation constants (K_i values) of the corresponding drugs for DTG site 1 in the guinea pig brain reported

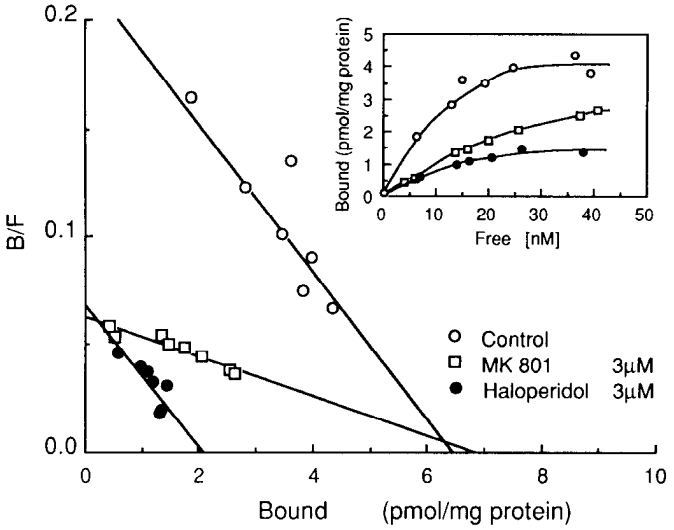

Figure 3. Scatchard plot of ^{3}H-TCP intact cell binding of primary cultured neuron with or without 3 μM of haloperidol or (±)MK 801. The results are the average of triplicate determinations from a representative experiment that was performed three times. The values for the density of binding sites (B_{max}) were 6.62 ± 0.35 pmol/mg protein (*control*) (N = 7), 1.59 ± 0.27 pmol/mg protein (*with haloperidol*) (N = 5), and 7.61 ± 0.61 pmol/mg protein [*with (±)MK 801*] (N = 3), and those for the equilibrium dissociation constant (K_d) were 25.8 ± 3.4 nM (*control*), 25.5 ± 7.3 nM (*with haloperidol*), and 62.6 ± 12.3 nM [*with (±)MK 801*].

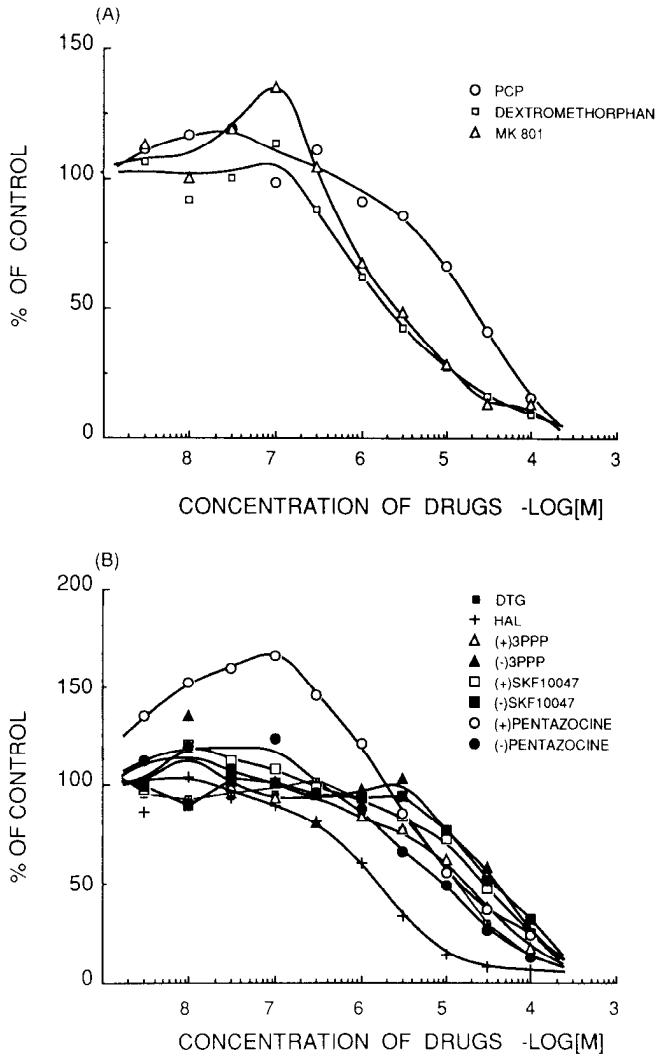

Figure 4. Competition curves of ^{3}H-TCP (5 nM) binding of intact neuronal cells by increasing concentrations of known NMDA receptor-ion channel complex blockers, including PCP, dextromethorphan, and MK 801 (*A*) or σ ligands including DTG, haloperidol, 3-PPPs, SKF 10047s, and pentazocines (*B*). Each value shows the average of four to nine separate experiments performed in triplicate.

by Rothman et al. (1991). There was a good correlation between the IC_{50} and the K_i values (r = 0.90) (Fig. 5).

Discussion

The present findings were, in most respects, consistent with the observations obtained from the ^{3}H-TCP binding studies using membrane preparations. However, ^{3}H-TCP intact cell binding reached equilibrium faster than ^{3}H-TCP binding to the membrane preparation (Hori et al., 1990). The findings that L-glutamate and glycine stimulated ^{3}H-TCP binding to extensively washed rat cortical membranes (Hori et al., 1991), suggest acceleration of ^{3}H-TCP intact cell binding by endogenous amino acids, such as L-glutamate and glycine, derived from the intact cells in the assay medium. This view is supported by our present observations not only that the addition of 0.5 mM of L-glutamate did not stimulate the ^{3}H-TCP binding to the intact cells, but also that a selective and competitive NMDA receptor antagonist, APV, decreased ^{3}H-TCP intact cell binding in the absence of both L-glutamate and Mg^{2+}

Table 1. Comparisons of IC_{50} values in inhibiting ^{3}H-TCP binding obtained from studies using intact neuronal cells or membrane preparation of rat brain

Drugs	IC_{50} from intact cells	IC_{50} from membranes
Dextromethorphan	1.760 ± 0.410 (7)	n.d.
(±)MK 801	2.025 ± 0.445 (8)	0.008 ± 0.003 (4)
PCP	12.478 ± 2.354 (4)	0.025 ± 0.003 (4)
Haloperidol	1.863 ± 0.391 (7)	18.880 ± 1.410 (4)
(+)Pentazocine	4.572 ± 0.608 (4)	n.d.
(−)Pentazocine	7.147 ± 3.163 (4)	n.d.
DTG	10.207 ± 1.443 (6)	4.747 ± 0.988 (4)
(+)SKF10047	14.654 ± 3.052 (9)	0.274 ± 0.093 (4)
(+)3-PPP	17.581 ± 4.519 (6)	n.d.
(−)SKF10047	20.476 ± 4.800 (9)	n.d.
(−)3-PPP	27.072 ± 3.140 (5)	n.d.

Values (μM) indicate mean ± SEM of four to nine independent experiments, each conducted in triplicate. n.d., not determined.

Mg^{2+} inhibits ^{3}H-TCP binding noncompetitively (Vignon et al., 1982). In addition, electrophysiological and biochemical studies have shown that Mg^{2+} recognition sites exist in the ion channel and an inhibitory effect of Mg^{2+} on ^{3}H-TCP binding is exerted through these Mg^{2+} recognition sites (Mayer et al., 1984; Nowak et al., 1984; Foster and Fagg, 1987; Kemp et al., 1987; Reynolds and Miller, 1988). The present finding that Mg^{2+} concentrations higher than 1 mM inhibited ^{3}H-TCP intact cell binding is in good agreement with the previous findings obtained in binding studies using either well-washed or crude membrane preparations. The above-mentioned findings, i.e., that L-glutamate did not stimulate ^{3}H-TCP binding to the intact cells and

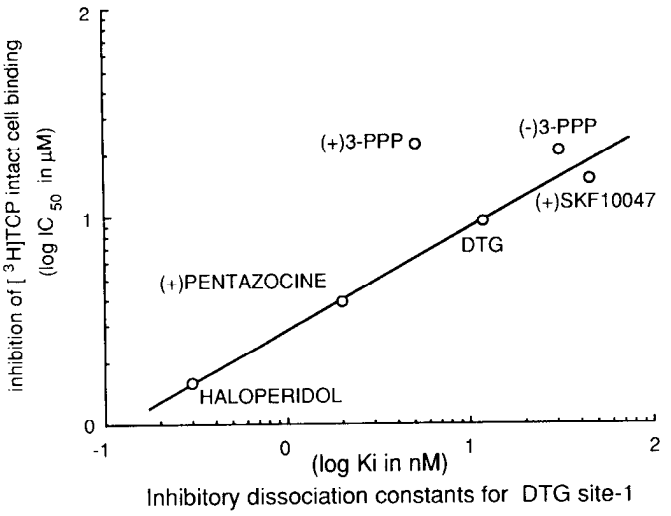

Figure 5. Correlation between the DTG site 1 binding potency from another laboratory's studies (Rothman et al., 1991) and potency in inhibiting ^{3}H-TCP intact cell binding of primary cultured neuronal cells.

that APV decreased ^{3}H-TCP intact cell binding, suggest that ^{3}H-TCP binding to primary cultured neuronal cells reflects *in vivo* ^{3}H-TCP binding to the NMDA receptor-ion channel complex.

Noncompetitive NMDA receptor antagonists, MK 801 and PCP, inhibited the specific binding of ^{3}H-TCP to the intact cells. However, the inhibitory effects of these two antagonists on ^{3}H-TCP intact cell binding were much weaker than their inhibitory effects on ^{3}H-TCP binding to membrane preparations. Previously, we reported that monovalent cations, Na^+ and K^+, inhibited ^{3}H-TCP binding to well-washed membranes in a concentration-dependent manner (Hatta et al., 1991). Considering that intact cell binding occurred in LMC, NaCl (150 mM) and KCl (5.6 mM) in the LMC may have inhibited ^{3}H-TCP intact cell binding more strongly than ^{3}H-TCP binding to membrane preparations in 5 mM Tris-HEPES buffer alone.

In most previous ligand-binding studies using membrane preparations, specific σ ligands have been reported to display low affinity for sites labeled by ^{3}H-TCP (Quirion et al., 1987; Deutsch et al., 1988; Church and Lodge, 1990; Sanger and Joly, 1991). The most pronounced difference between ^{3}H-TCP intact cell binding and ^{3}H-TCP binding to membrane preparations is that even though they have low affinity for the NMDA receptor-ion channel complex, σ ligands, especially haloperidol, inhibited ^{3}H-TCP intact cell binding in a noncompetitive manner. Furthermore, it should be noted that although its underlying mechanism is not clear, displacement of ^{3}H-TCP from its specific binding sites by haloperidol was somewhat slower than that by PCP. These findings suggest that σ ligands could indirectly modulate the NMDA receptor-ion channel complex on the intact cells. In other words, it seems possible that σ ligands indirectly modulate the NMDA receptor-ion channel complex through σ binding sites *in vivo*. In addition, the present observations that, although (±)MK 801 and (+)pentazocine stimulated ^{3}H-TCP intact cell binding at low concentrations, the IC_{50} values of several test drugs for ^{3}H-TCP binding were closely correlated with the K_i values of the corresponding drugs for ^{3}H-DTG site 1, which is regarded as the same as the DM1/σ1 site (Rothman et al., 1991) provides supporting evidence for our above-mentioned view and, furthermore, suggests that σ ligands indirectly act on the NMDA receptor-ion channel complex through σ1 sites.

Recently, electrophysiological studies (Chen and Huang, 1991, 1992) have shown that protein kinase C (PKC) potentiates the NMDA response by increasing the probability of channel openings and by reducing the voltage-dependent Mg^{2+} block of the ion channel of the NMDA receptor-ion channel complex. Furthermore, Kitamura et al. (1993) have reported that pretreatment with purified type II of PKC and Ca^{2+}/calmodulin-dependent PK-II (CaMK-II)-catalyzed phosphorylation enhanced the L-glutamate–induced increase of ^{3}H-MK-801 binding, suggesting that PKC-II and/or CaMK-II induce the phosphorylation of the channel domain of the NMDA receptor-ion channel complex.

Bowen et al. (1988, 1989) have shown that σ ligands, such as (+)pentazocine, DTG, and haloperidol, inhibit carbachol-stimulated phosphoinositide (PI) turnover in a dose-dependent manner, suggesting that σ ligands block the activation of phospholipase C induced by G-protein–coupled muscarinic receptors. On the other hand, PKC activation is often associated with PI hydrolysis, that is, the products of PI hydrolysis, diacylglycerols, cause PKC activation (Takai et al., 1979; Kishimoto et al., 1980). These findings suggest that σ ligands exert inhibitory effects on channel openings of the NMDA receptor-ion channel complex by inhibition of phosphorylation through a mechanism(s) in which phospholipase C is involved. However, further studies are necessary to elucidate the mechanisms of the inhibitory effects of σ ligands on the NMDA receptor-ion channel complex.

In conclusion, the present study showed that the σ ligands tested inhibited ^{3}H-TCP intact cell binding and that the IC_{50} values of several test drugs for ^{3}H-TCP binding were closely correlated with the K_i values of the corresponding drugs for ^{3}H-DTG site 1, which is regarded as the σ1 site. Based on these findings, we postulated that σ ligands indirectly modulate the NMDA receptor-ion channel complex through the σ1 site *in vivo*.

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