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Uncompetitive inhibition of [^{3}H]1,3-di-*o*-tolyl-guanidine-defined σ binding sites by desipramine, propranolol and alprenolol in rat brain

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Abstract

Desipramine, imipramine, clomipramine, (−)-propranolol, (−)-alprenolol, (±)-pentazocine and risperidone caused a concentration-dependent inhibition of 6 nM [^{3}H]DTG (1,3-di-*o*-tolylguanidine)-defined sigma (σ) binding with K_i values of about 0.5–2.5 μM in well-washed homogenates obtained from rat cerebral cortex. The saturation studies revealed that the inhibition by desipramine (1–4 μM), (−)-propranolol (1 μM) and (−)-alprenolol (3 μM) resulted from a reduction of the B_{max} value without alteration of the K_d of [^{3}H]DTG binding to the cortex or hippocampus. In contrast, imipramine, (±)-pentazocine, clomipramine and risperidone competitively attenuated the cortical or hippocampal [^{3}H]DTG binding. These findings demonstrate the uncompetitive inhibition of [^{3}H]DTG binding by neuroactive drugs, thereby providing further support for the possible multiple regulation of cerebral σ receptors. © 1997 Elsevier Science B.V.

Keywords: DTG (1,3-di-*o*-tolylguanidine); σ Binding site; Desipramine; Propranolol; Alprenolol; Cerebral cortex, rat

1. Introduction

Sigma (σ) binding sites have been implicated in the pathophysiology of psychomotor disturbances because a wide variety of centrally acting drugs exhibit high to moderate affinity for the σ sites defined by various radioactive ligands such as [^{3}H]1,3-di-*o*-tolylguanidine ([^{3}H]DTG), [^{3}H](+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ([^{3}H]3PPP) and [^{3}H](+)-pentazocine (for review, see Walker et al., 1990). In support of this hypothesis, local application of sigma ligands to the red nucleus has been shown to produce motor actions such as torticollis in rats (Walker et al., 1990 for a review). More recent biochemical analysis of post-mortem human brains has indicated that there are significant changes in σ binding activity in discrete brain areas of schizophrenic patients (Weissman et al., 1991; Shibuya et al., 1992; Helmeste et al., 1996). These observations, together with the interactions between the σ sites and neurotransmitter or neuromodulator systems (Su, 1991; Debonnel and de Montigny, 1996), suggest that further investigation of the mechanism underlying the actions of psychoactive agents on brain σ sites would aid the development of novel pharmacotherapy and the understanding of the molecular basis of psychomotor symptoms.

We previously showed that repeated administration of antidepressants including imipramine and fluoxetine, but not desipramine, reduced the number of [^{3}H]DTG defined σ binding sites in rat striatum, hippocampus and cerebral cortex, although these drugs have a similar potency in inhibiting [^{3}H]DTG binding in in vitro preparations (Shirayama et al., 1993). The *p*-chlorophenylalanine-reversible nature of this reduction of σ binding indicates the involvement of serotonergic transmission in the chronic effects of imipramine and fluoxetine (Shirayama et al., 1993). However, the differences in in vitro effects between the two antidepressants and desipramine were not fully characterized. To obtain insight into the possible differences, we studied the mode of inhibition by desipramine of [^{3}H]DTG binding in well-washed homogenates of the cerebral cortex and hippocampus of the rat in comparison with

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other neuroactive drugs including clomipramine, fluoxetine, propranolol and alprenolol.

2. Materials and methods

2.1. Animals and brain dissection

Male Wistar rats (St strain, Shizuoka Laboratory Animal, Japan) weighing 180–250 g were used. The animals were housed at 22.0 ± 0.5°C in a humidity controlled room under a 12 h light/dark cycle and had free access to food and water. For the preparation of the homogenates from the brain tissues, the rats were killed by decapitation and the hippocampus and cerebral cortex were dissected out in the cold, frozen on solid CO_2 and stored at −80°C until biochemical analysis.

2.2. [^{3}H]DTG binding assay

[^{3}H]DTG (39.1–60.0 Ci/mmol, New England Nuclear (Boston, MA, USA), binding to the haloperidol-sensitive σ site, was evaluated according to the method described previously (Shirayama et al., 1993, 1994). Briefly, the brain tissues were homogenized in 20 volumes of ice cold 50 mM Tris–HCl buffer (pH 7.7 at 25°C) with a Brinkmann polytron (setting 6, 30 s). An excess volume of fresh 50 mM Tris–HCl buffer was added to the homogenates; the material was centrifuged at 40 000 × g for 20 min at 4°C. The resultant pellets were then resuspended in 30 volumes of fresh buffer, using the polytron (setting 5, 10 s), an excess volume of fresh buffer was added and the suspensions were recentrifuged at 40 000 × g for 20 min at 4°C. This procedure was repeated once more before the homogenates were suspended in 30 volumes of 50 mM Tris–HCl buffer (pH 8.0) for use. The suspensions (1.5–2.0 mg protein/ml) were frozen at −80°C until use.

[^{3}H]DTG binding assays were performed at 25°C for 90 min in a final volume of 0.5 ml. The incubation medium consisted of 0.5 ml of 50 mM Tris–HCl buffer (pH 8.0) containing 0.15–0.20 mg of membrane protein, various concentrations of [^{3}H]DTG (39–60 Ci/mmol, NEN) and unlabeled drugs. Non-specific binding was defined in the presence of 10 μM unlabeled haloperidol. Saturation studies were performed with the homogenates and increasing concentrations (eight concentrations ranging from 10 to 210 nM or routinely five concentrations ranging from 10 to 110 nM) of [^{3}H]DTG. For competition experiments, the reaction medium contained cerebral homogenates, 3 or 6 nM [^{3}H]DTG and various concentrations of unlabeled compounds. We confirmed that there was no change in pH in the presence of any drugs tested in the assay medium.

The binding reaction was terminated by rapid filtration through Whatmann GF/B glass fiber filters presoaked in 0.5% polyethyleneimine (pH 8.0), followed by three washings with ice-cold 50 mM Tris–HCl buffer (pH 8.0 at 25°C) and extraction in Aquasol-2 (NEN) scintillation cocktail. The bound radiolabeled ligand was measured by liquid scintillation spectroscopy. Protein content was measured according to the method of Lowry et al. (1951).

2.3. Data and statistical analysis

The concentration of test compounds which produced 50% inhibition (IC_{50}) of [^{3}H]DTG binding was determined from a log-probability scale. Inhibition constant (K_i) values and Hill coefficients (n_H) of competing drugs at the σ site were estimated according to the equation of Cheng and Prusoff (1973) and Hill plot analysis, respectively. The apparent dissociation constant (K_d) and maximal binding (B_{max}) values were calculated by linear regression analysis of Scatchard plots.

For comparison of the mean values among three groups, statistical evaluations were made using a one-way analysis of variance (ANOVA) followed by a multiple comparison test (Scheffé's method).

2.4. Chemicals

Desipramine–HCl and haloperidol were kind gifts from Ciba Geigy Japan (Tokyo, Japan) and Dainippon Pharmaceutical (Suita, Japan), respectively. Risperidone was kindly donated by Janssen Kyowa (Tokyo, Japan). The other chemicals used here were of ultrapure quality and were obtained from commercial sources.

3. Results

We previously reported (Shirayama et al., 1993) that desipramine, fluoxetine, clomipramine and desipramine

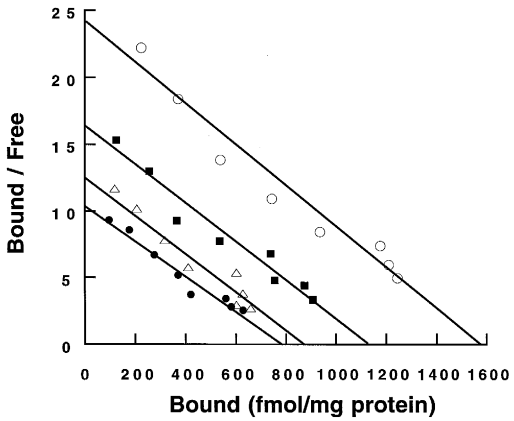

Fig. 1. Scatchard plot of saturation data of specific [^{3}H]DTG binding to rat cortical P2 fraction in the absence (○) and presence of desipramine (1 μM (■), 2 μM (△), 4 μM (●)). Binding assays were performed as described in Section 2. Brain homogenates were incubated with eight concentrations of [^{3}H]DTG (10–210 nM) at 25°C in the presence or absence of 10 μM unlabeled haloperidol. Each point is the mean of 3–7 determinations.

Table 1
Saturation binding parameters of [^{3}H]DTG binding to rat cortical and hippocampal homogenates in the absence and presence of desipramine, clomipramine, risperidone, (+)-MK801, CGS19755 or (−)-propranol

Treatment		K_d (nM)	B_{max} (fmol/mg protein)	n
Cortex				
Control		44±2	1491±44	9
Desipramine	2 μM	55±3	918±60 [a]	5
Risperidone	2 μM	93±8 [a]	1548±153	3
Control		42±5	1383±30	5
(+)-MK801	1 μM	44±1	1329±80	3
CGS19755	1 μM	44±8	1315±60	3
(−)-Propranolol	1 μM	56±10	1031±37 [a]	3
Hippocampus				
Control		69±6	1040±28	8
Desipramine	2 μM	69±14	493±13 [a]	3
Clomipramine	1 μM	163±15 [a]	1098±100	3

Binding assays were performed as described in Section 2. Homogenates were incubated with five concentrations of [^{3}H]DTG (10–110 nM) at 25°C in the presence or absence of 10 μM unlabeled haloperidol. The results are the means with S.E.M. of data obtained from 3–9 independent experiments.
[a] $P < 0.01$ as compared to respective controls.

produce a concentration-related inhibition of [^{3}H]DTG defined σ binding sites in the rat cortical homogenates with K_i values ranging from 500–2500 nM. In the present study, a novel neuroleptic drug, risperidone and β-adrenoceptor antagonists, propranolol and alprenolol, were further found to attenuate [^{3}H]DTG binding to the cerebral cortex with the following K_i and Hill coefficient values: risperidone, $K_i = 2361 \pm 347$, $n_H = 0.95 \pm 0.12$ ($n = 5$); (−)-propranolol, $K_i = 2243$, $n_H = 0.80$ ($n = 2$); (−)-alprenolol, $K_i = 2144$, $n_H = 0.84$ ($n = 2$). Non-competitive ((+)-dizocilpine: (+)-MK-801) and competitive (*cis*-4-phosphonomethyl-2-piperidine carboxylic acid: CGS19755) antagonists of the *N*-methyl-D-aspartate (NMDA) receptor failed to compete with cortical [^{3}H]DTG binding even at a high concentration of 10 μM (data not shown).

Table 2
Saturation binding parameters of [^{3}H]DTG binding to rat cortical homogenates in the absence and presence of desipramine, (−)-propranolol or (−)-alprenolol

Treatment		K_d (nM)	B_{max} (fmol/mg protein)	n
Cortex				
Control		63±5	1581±54	7
Desipramine	1 μM	70±8	1129±85 [a]	3
Desipramine	2 μM	63±5	879±34 [a]	6
Desipramine	4 μM	69±4	788±67 [a]	4
Control		62±6	1628±68	4
(−)-Propranolol	1 μM	68±3	1233±38 [a]	3
(−)-Alprenolol	3 μM	66±8	817±89 [a]	3

Binding assays were performed as described in Section 2. Homogenates were incubated with eight concentrations of [^{3}H]DTG (10–210 nM) at 25°C in the presence or absence of 10 μM unlabeled haloperidol. The results are the means with S.E.M. of data obtained from 3–7 independent experiments.
[a] $P < 0.01$ as compared to respective controls.

The saturation experiments of [^{3}H]DTG (10–110 or 10–210 nM) binding to the cortical and hippocampal σ sites in the presence of various drugs revealed that desipramine (1–4 μM) caused a concentration-dependent decrease in the B_{max} value without affecting the K_d value of the radioligand binding (Fig. 1, Tables 1 and 2). Similar effects on [^{3}H]DTG binding were observed in the presence of (−)-alprenolol (3 μM) or (−)-propranolol (1 μM)(Tables 1 and 2). By contrast, imipramine, clomipramine and risperidone increased the K_d with no effect on the B_{max} (Table 1). Neither MK-801 nor CGS19755 changed the binding parameters of the cortical σ sites (Table 1).

4. Discussion

The present study is the first to demonstrate that [^{3}H]DTG binding to cortical σ sites can be uncompetitively inhibited by neuroactive drugs such as desipramine and β-adrenergic antagonists with relatively high potency at low micromolar concentrations. This phenomenon does not appear to be due to an artifact because, in agreement with our previous report (Shirayama et al., 1993), imipramine caused competitive inhibition of the binding under the same conditions. Clomipramine and risperidone are also shown to inhibit competitively [^{3}H]DTG binding.

We selected [^{3}H]DTG as a radioligand because DTG shows no substantial cross-reaction to the dopamine, serotonin and phencyclidine (PCP) receptor sites, for which other radiolabeled σ ligands show high affinity (Largent et al., 1984; Tam and Cook, 1984; Weber et al., 1986; Tam et al., 1992). However, [^{3}H]DTG binding does not discriminate between σ_1 and σ_2 subtypes (Quirion et al., 1992). We, therefore, cannot define which σ subtype was involved in the desipramine- or β-antagonist-induced changes in [^{3}H]DTG binding under our assay conditions.

The mechanism underlying the uncompetitive inhibition of [^{3}H]DTG binding is still unclear. Desipramine could modify brain σ binding sites through its interaction with the norepinephrine uptake site (Richelson and Pfenning, 1984). However, this idea appears to conflict with the fact that a competitive inhibitor of [^{3}H]DTG binding, imipramine, also has a high affinity for the monoamine uptake site (Richelson and Pfenning, 1984). Because desipramine causes a blockade of NMDA-induced Ca^{2+} influx (Reynolds and Miller, 1988; Sernagor et al., 1989) and a complete inhibition of specific [^{3}H]MK-801 binding to the NMDA receptor channel with a K_i value around 4 μM (Reynolds and Miller, 1988; Bakker et al., 1991), the NMDA blocking action could be associated with the attenuating effects of desipramine on [^{3}H]DTG binding. A mutual interaction between NMDA and σ receptors has

indeed been reported in brain tissues (Malouf et al., 1988; Monnet et al., 1990; Rao et al., 1990; Yamamoto et al., 1995). This assumption, however, seems unlikely because of the present observation that both the competitive NMDA receptor antagonist CGS19755 and the non-competitive NMDA receptor antagonist (+)-MK-801 produced no change in the binding parameters of [^{3}H]DTG (Table 1). It is also possible that the desipramine-induced reduction of the σ site might be mediated by its moderate action at the β-adrenoceptor (Richelson and Pfenning, 1984). Although some β-adrenoceptor antagonists mimicked the ability of desipramine to attenuate uncompetitively [^{3}H]DTG binding to the cortical homogenates (Tables 1 and 2), the above hypothesis does not explain the apparent discrepancy between the rank order potency of these agents as β-blockers (Richelson and Pfenning, 1984) and as inhibitors of the σ binding site (Tables 1 and 2).

The cerebral σ site may be allosterically modified by desipramine, (−)-propranolol and (−)-alprenolol through undefined binding domains on the σ macromolecule. The possible existence of multiple regulatory sites of the σ receptor is also supported by the observation that [^{3}H]3-PPP (Paul et al., 1990) and [^{3}H]SKF-10047 (*N*-allylnormetazocine) (Tsao and Su, 1996) binding to σ sites can be non-competitively inhibited by polyamines such as spermine, spermidine, cadaversine and putrescine, and by an endoplasmic reticulum Ca^{2+} modulator heparin, respectively. Whatever the mechanisms, the multiple regulation of σ sites might play a functional role in mammals, because (1) phenytoin and ropizine have been shown to facilitate [^{3}H]dextromethorphan and [^{3}H](+)-3-PPP binding to σ sites in the guinea pig brain (Musacchio et al., 1989), and (2) propranolol enhances the emetic effects of DTG without producing emesis by itself in the guinea pig (Hudzik et al., 1993).

The uncompetitive interaction of desipramine, (−)-propranolol and (−)-alprenolol with cerebral σ sites could be involved in their central actions, although the exact functional roles of the interaction are uncertain. The distinct influence of antidepressants including imipramine, clomipramine, desipramine and fluoxetine on cerebral σ sites (Shirayama et al., 1993; the present paper) might result in certain differences in their clinical effects on psychomotor functions. To extend our knowledge to the drug development and to the pathophysiology of neuropsychiatric disorders, the possible multiregulation of the σ receptors should be explored in the appropriate systems for the expression of cloned σ receptor genes (Hanner et al., 1996).

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