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Short communication

Interactions of selective serotonin reuptake inhibitors with subtypes of σ receptors in rat brain

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Abstract

The interactions of selective serotonin reuptake inhibitors and tricyclic antidepressants with subtypes of σ receptors were investigated. The rank order of affinity (K_i values) from competition experiments of [^{3}H](+)-pentazocine binding to σ_1 sites was: fluvoxamine > sertraline > *S*(+)-fluoxetine > (±)-fluoxetine > citalopram > imipramine > paroxetine > desipramine > *R*(−)-fluoxetine > (±)-norfluoxetine. The K_i values of all drugs for σ_2 sites were more than 1000 nM. Furthermore, all drugs were more potent at σ_1 sites than at σ_2 sites. These findings suggest that σ receptors (σ_1 site) may play, in some way, a role in the actions of selective serotonin reuptake inhibitors.

Keywords: 5-HT (5-hydroxytryptamine, serotonin) reuptake inhibitor; σ Receptor, subtype; Binding; Brain, rat

1. Introduction

The selective serotonin reuptake inhibitors, such as fluoxetine, paroxetine, fluvoxamine, sertraline and citalopram, have been shown to be effective in the treatment of major depression. Furthermore, it has been demonstrated that these drugs are the most efficacious treatment of diseases such as obsessive compulsive disorders, bulimia nervosa, panic disorder and obesity (reviews by Fuller, 1992; Blier and De Montigny, 1995; Wong et al., 1995).

σ Receptors have been implicated in various pharmacological and physiological functions in the brain (reviews by Walker et al., 1991; Su, 1993). It has been reported that σ receptors are altered by repeated treatment with imipramine or fluoxetine (Shirayama et al., 1993), and that administration of a selective σ receptor ligand *N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride (NE-100) might regulate the 5-HT$_{2A}$ receptor (Narita et al., 1995), suggesting that there is an interaction between σ receptors and serotonergic neurons in the brain. Moreover, it is reported that sertraline inhibits [^{3}H](+)-3-(3-hydroxyphenyl)-*N*-propylpiperidine (3-PPP) binding to σ receptors with high affinity (Schmidt et al., 1989). At present, two subtypes (σ_1 and σ_2) of σ receptors have been proposed (Quirion et al., 1992; Bowen et al., 1993). In this study, we examined the effects of several serotonin reuptake inhibitors and typical tricyclic antidepressants (imipramine and desipramine) on the subtypes of σ receptors in rat brain. Furthermore, fluoxetine has been marketed as a racemic mixture containing equal amounts of *R*(−)- and *S*(+)-enantiomers. Both enantiomers and the major metabolite norfluoxetine have been shown to inhibit serotonin reuptake with moderate to high potency (Wong et al., 1993). Therefore, we also examined the effects of norfluoxetine and enantiomers of fluoxetine on the two σ receptor subtypes.

2. Materials and methods

2.1. Materials

The following drugs were obtained from the following sources: fluoxetine, *R*(−)-fluoxetine, *S*(+)-fluoxetine and (±)-norfluoxetine (Eli Lilly and Company, Indianapolis, IN, USA); paroxetine (SmithKline Beecham Pharmaceuticals, West Sussex, UK); citalopram (H. Lundbeck, Copenhagen-Valby, Denmark); sertraline (Pfizer, Groton, CT, USA); fluvoxamine (Solvey-Meiji, Tokyo, Japan); (+)-pentazocine (Taisho Pharmaceutical Co., Ohmiya, Saitama, Japan); imipramine, desipramine and haloperidol (Wako

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Pure Chemical Industries, Tokyo, Japan); and [^{3}H](+)-pentazocine (1417 GBq/mmol) and [^{3}H]1,3-di-*o*-tolylguanidine (DTG) (1302 GBq/mmol) (DuPont/New England Nuclear, Boston, MA, USA). Other chemicals were purchased commercially and were analytical grade.

2.2. *Receptor binding assay*

Binding assays for subtypes of σ receptors were performed according to the method published previously (Hashimoto and London, 1995). Male Sprague-Dawley rats (250–300 g) were killed by decapitation, and the brains were rapidly removed. The brains were homogenized in 20 volumes of ice-cold 50 mM Tris-HCl (pH 8.0 at 25°C) with a Kinematica Polytron homogenizer (Lucerne, Switzerland) at setting 5 for 30 s. The homogenate was centrifuged at 48000 × *g* for 10 min (4°C). The resulting pellet was resuspended in the buffer and recentrifuged. This procedure was repeated once more. The final pellet was suspended in 20 volumes of 50 mM Tris-HCl (pH 8.0 at 25°C). For assays of binding to σ_1 sites, aliquots of crude membranes (approximately 350–400 μg protein) were incubated with [^{3}H](+)-pentazocine (5 nM) and 50 mM Tris-HCl (pH 8.0 at 25°C) in a final volume of 0.5 ml for 2 h at 25°C. For assays of binding to σ_2 sites, aliquots of crude membranes (approximately 350–400 μg protein) were incubated with [^{3}H]DTG (5 nM), 1 μM (+)-pentazocine (to mask σ_1 site) and 50 mM Tris-HCl (pH 8.0 at 25°C) in a final volume of 0.5 ml for 2 h at 25°C. After addition of 4 ml of ice-cold buffer, the membranes were rapidly filtered, using a Brandell 24-channel cell harvester (Biochemical Research Laboratories, Gaithersburg, MD, USA), through Whatman GF/B filters pretreated with 0.5% polyethyleneimine for at least 2 h. The filters were washed three times with 4 ml of ice-cold buffer. The radioactivity trapped by the filters was determined by a liquid scintillation counter (Packard, Tri-Carb 2200, CA, USA). Non-specific binding was estimated in the presence of 10 μM haloperidol. The concentrations of protein were measured colorimetrically after reaction with a concentrated dye reagent Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA, USA), using bovine serum albumin as the standard. Values of the inhibitory affinity constant ($K_i = IC_{50}/(1 + [L]/K_d)$, where [L] was the concentration of radioligand used, IC_{50} was the concentration that resulted in 50% inhibition of specific binding and K_d was the dissociation constant, were calculated using the EBDA/LIGAND programs (Biosoft, UK).

3. Results

The data for σ receptor binding are shown in Table 1. The rank order of potency of drugs in competing with [^{3}H](+)-pentazocine binding to σ_1 sites was as follows: fluvoxamine > sertraline > *S*(+)-fluoxetine > (±)-fluoxetine > citalopram > imipramine > paroxetine > desipramine > *R*(−)-fluoxetine > (±)-norfluoxetine. All drugs used in this study had an affinity for the σ_2 sites of more than 1000 nM. All drugs were more selective for σ_1 sites than for σ_2 sites, as shown in Table 1. Fluvoxamine was the most selective ligand for σ_1 sites, as the ratio of the K_i values obtained in binding to σ_2 sites vs. σ_1 sites was 234. *S*(−)-Fluoxetine was 18 times more potent than *R*(+)-fluoxetine, K_i values being 120 and 2180 nM, respectively. (±)-Norfluoxetine, a metabolite of fluoxetine, was about 10 times less potent than the parent compound (±)-fluoxetine at σ_1 sites, as shown in Table 1.

Table 1
Affinities of selective serotonin reuptake inhibitors and tricyclic antidepressants for the subtypes of σ receptors

Drugs	K_i (nM)		K_i ratio
	σ_1	σ_2	σ_1/σ_2
Fluvoxamine	36	8439	234
Sertraline	57	5297	93
S(+)-Fluoxetine	120	5480	46
(±)-Fluoxetine	240	16100	68
Citalopram	292	5410	19
Imipramine	343	2107	6
Paroxetine	1893	22870	12
Desipramine	1987	11430	6
R(−)-Fluoxetine	2180	24100	11
(±)-Norfluoxetine	2377	34630	19

Assays were carried out under conditions described in Materials and methods. Ten concentrations of unlabeled compound ranging from 3 nM to 100000 nM were incubated with rat brain membranes and 5 nM [^{3}H](+)-pentazocine (σ_1 sites) or 5 nM [^{3}H]DTG in the presence of 1 μM (+)-pentazocine (σ_2 sites). The values are the means of three experiments done in duplicate, the S.E.M. of which were less than 10%.

4. Discussion

Several selective serotonin reuptake inhibitors (fluvoxamine, sertraline, (±)-fluoxetine, citalopram and (+)-fluoxetine) and the tricyclic antidepressant imipramine have moderate to high affinity for σ_1 sites in rat brain. The potent selective serotonin reuptake inhibitor paroxetine had low affinity for σ_1 sites. These data are consistent with previous finding (Schmidt et al., 1989) obtained with [^{3}H](+)-3-PPP as a radioligand. All drugs used in this assay were more potent at σ_1 sites than at σ_2 sites. Fluvoxamine is a potent and selective ligand for σ_1 sites, as the ratio of the K_i values obtained in binding to σ_2 sites vs. σ_1 sites was 234. Wong et al. (1993) reported that, like (±)-fluoxetine, *R*(−)- and *S*(+)-enantiomers of fluoxetine and (±)-norfluoxetine inhibited serotonin reuptake with almost equipotency. However, no correlation between the potency of drugs for σ_1 sites and the potency of drugs for inhibition of serotonin uptake was shown. Thus, the potency of the drugs for inhibition of serotonin uptake was not correlated with the potency of the drugs for σ_1 sites.

Recent data suggests that dysfunction of serotonergic function may be involved in the pathophysiology of schizophrenia, and that pharmacological agents for this disease exert their therapeutic effects through serotonergic mechanisms (review by Breier, 1995). In a double-blind study, fluvoxamine and placebo were added to conventional neuroleptics in the treatment of schizophrenic patients. It was reported that fluvoxamine was superior to placebo for negative symptoms (see Breier, 1995). Furthermore, it has been reported that fluoxetine added to neuroleptics is superior to placebo for negative symptoms (Goff et al., 1990; Breier, 1995). Thus, these data suggest that selective serotonin reuptake inhibitors such as fluvoxamine and fluoxetine may be effective for negative symptoms in schizophrenic patients. However, the mechanism(s) underlying the effects of these drugs in the treatment of schizophrenia are currently unknown. There is evidence that σ receptors may play a role in psychosis and might be involved in the pathophysiology of schizophrenia (see Walker et al., 1991; Su, 1993). Furthermore, it has been suggested that σ receptors are the target for the development of atypical antipsychotic drugs that do not produce extrapyramidal side effects. Taken together, it seems that the antipsychotic effects of selective serotonin reuptake inhibitors such as fluvoxamine and fluoxetine for the negative symptoms in schizophrenic patients may be, in part, due to an interaction with σ receptors (σ_1 sites) in the brain, although the relative contributions of these drugs on serotonin reuptake inhibition and σ_1 sites have not been evaluated. Further detailed studies on this possibility are necessary.

In conclusion, these results suggest that selective serotonin reuptake inhibitors such as fluvoxamine, sertraline, fluoxetine and citalopram have moderate to high affinity for σ_1 sites, but not σ_2 sites, and that σ_1 sites may, in some way, play a role in the pharmacological effects of selective serotonin reuptake inhibitors in the brain.

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