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Short communication

Adaptation of cortical NMDA receptors by chronic treatment with specific serotonin reuptake inhibitors

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Abstract

Glycine displaces [^{3}H]CGP-39653 ([^{3}H]D,L-(E)-2-amino-4-propyl-5-phosphono-3-pentenoic acid) binding to the glutamate recognition site with both high and low affinity. We reported previously that chronic treatment with antidepressants reduced the proportion of high to low affinity sites, or, even eliminated the high affinity sites in case of citalopram. Here, we compared the effects of citalopram with another serotonin specific reuptake inhibitor, fluoxetine on this measure. Chronic administration of citalopram or fluoxetine eliminated high affinity glycine-displaceable [^{3}H]CGP-39653 binding to the mouse cortex in 78 and 56% of animals, respectively, indicating that selective serotonin reuptake inhibitors produce qualitatively similar adaptive changes at NMDA receptors, that differ from other antidepressants in this neurochemical measure. © 1998 Elsevier Science B.V.

Keywords: Citalopram, chronic; Fluoxetine, chronic; NMDA receptor; Adaptation; Cortex

1. Introduction

Citalopram and fluoxetine are selective serotonin reuptake inhibitors, a class of compounds that are safer and better tolerated by patients compared to tricyclic antidepressants (Montgomery, 1996). Recently, *N*-methyl-D-aspartate (NMDA) receptors have been implicated in both the behavioral and the adaptive neuronal response to antidepressant treatments (Skolnick et al., 1996; Shader et al., 1997). This family of ligand-gated ion channels possess multiple, allosterically coupled recognition sites for glutamate, glycine, polyamines and use-dependent channel blockers (Carter, 1992). Chronic, but not acute treatment with a wide range of clinically effective antidepressants produce adaptive changes in radioligand binding to NMDA receptors. This adaptation is manifested as: (1) a reduction in potency of glycine to inhibit [^{3}H]5,7-dichlorokynurenic acid binding to strychnine-insensitive glycine receptors and (2) a reduction in the proportion of high affinity glycine sites inhibiting the binding of [^{3}H]CGP-39653 ([^{3}H]D,L-(E)-2-amino-4-propyl-5-phosphono-3-pentenoic acid) to glutamate recognition sites (Nowak et al., 1993; Paul et al., 1993, 1994). Previous studies demonstrated that imipramine and electroconvulsive shock (Nowak et al., 1993; Paul et al., 1993) as well as pargyline and amitryptyline (Huang et al., 1997) produce a reduction in the proportion of high affinity glycine sites inhibiting [^{3}H]CGP-39653 binding. However, this population of high affinity glycine sites was virtually eliminated by chronic treatment with the specific serotonin reuptake inhibitor, citalopram (Nowak et al., 1996). The purpose of the present study was to determine if this remarkable effect of citalopram on [^{3}H]CGP-39653 binding was shared by another selective serotonin reuptake inhibitor, fluoxetine.

2. Materials and methods

2.1. Animals

Male albino Swiss mice (20–25 g) were housed in groups of 9–10 under standard laboratory conditions. All procedures were conducted according to NIH Animal Care and Use Committee guidelines and approved by the Ethical Committee of the Institute of Pharmacology. Citalopram (20 mg/kg i.p., Lundbeck A/S, Copenhagen) and

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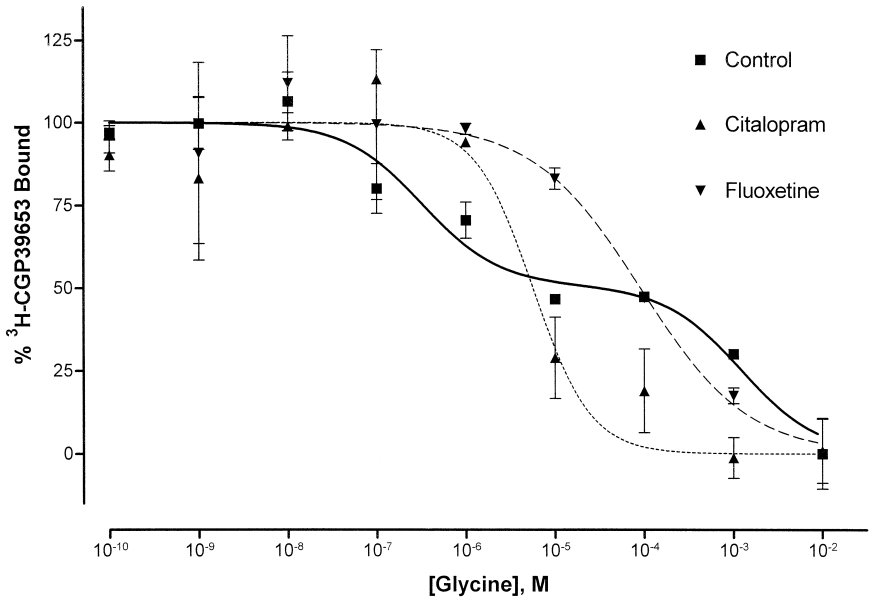

Fig. 1. Effect of chronic treatment with either citalopram or fluoxetine on glycine displacement of [^{3}H]CGP-39653 binding to mouse cortex. The curves shown are representative; see Table 1 for details. Data are expressed as percent specific binding.

fluoxetine (20 mg/kg i.p., Eli Lilly and Co., Indianapolis, IN) were administered once daily for 21 days. Animals were killed by decapitation 24 h after the last treatment, the brains were removed, neocortices dissected and placed on aluminum foil over solid CO_2. Tissues were stored at −70°C (2–6 weeks).

2.2. Radioligand binding assay

Radioligand binding assays were conducted essentially as previously described (Nowak et al., 1996). Initially, tissues were thawed in 50 volumes ice-cold 5 mM HEPES/4.5 mM Tris (HTS) buffer (pH 7.4), homogenized and centrifuged at $20\,000 \times g$ for 20 min. Tissue pellets were reconstituted in HTS buffer containing 10 mM EDTA and centrifuged. The resulting tissue pellet was resuspended in HTS buffer and stored at −70°C for at least 3 days. On the day of the assay, the frozen aliquot was thawed and washed three times with HTS. Assays consisted of membrane suspension (0.1–0.2 mg of protein), 5 nM [^{3}H]CGP-39653 (48.9 Ci/mmol; DuPont-NEN) and buffer and/or drugs to a final volume of 0.5 ml. Nonspecific binding was defined in the presence of 1 mM L-glutamate. Nine concentrations (10^{-2} to 10^{-10} M) of glycine were used to construct inhibition curves. Samples were incubated at 0–4°C for 1.5 h. Bound ligand was separated by vacuum filtration over Whatman GF/C filters and washed twice with 5 ml of ice-cold HTS buffer. The radioactivity was measured in a Beckman LS 3801 scintillation counter. All assays were performed in duplicate. Protein content was determined by the BSA protein assay kit (Sigma).

2.3. Data analysis

Data were analyzed using iterative curve fitting routines (GraphPAD Prism 2.0). A one-site model was assumed unless the sum of squares of the model was significantly reduced by employing a two-site model. Group differences were assessed using Fisher's exact probability test.

Table 1
Effect of chronic treatment with citalopram or fluoxetine on glycine inhibition of [^{3}H]CGP-39653 binding to mouse cortex

Treatment	Specific binding (fmol/mg protein)	Two sites				One site		2 sites/1 site
		High affinity, IC_{50} (nM)	%	Low affinity, IC_{50} (μM)	*n*	Affinity, IC_{50} (μM)	*n*	
Vehicle	128 ± 16	260 ± 76	52 ± 3	745 ± 153	10	—	0	10/0
Citalopram	104 ± 29	2, 85	52, 40	114, 287	2	24 ± 20	7	2/7[a]
Fluoxetine	147 ± 29	315 ± 231	50 ± 5	716 ± 222	4	215 ± 141	5	4/5[a]

Data represent the mean ± S.E.M.
n: number of mice per group.
[a] $P < 0.05$ versus control-vehicle treated mice (Fisher's exact test, 2 site/1 site).

3. Results

Consistent with previous findings (Sills et al., 1991; Mugnaini et al., 1993; Nowak et al., 1993, 1996) glycine displaced [^{3}H]CGP-39653 binding to cortical homogenates over a concentration range of 10^{-9}–10^{-2} M (Fig. 1). In control mice, displacement curves were best fit to a two site model (for pooled data $F(2, 6) = 38.43$, $P < 0.001$) in all subjects (Table 1). However, in citalopram treated mice, curves were best fit to a one-site model for 7 out of 9 subjects (for pooled data of 7 subjects, $F(2, 6) = 0.74$, $P = 0.52$) and to a two-site model for 2 subjects (for pooled data of 2 subjects, $F(2, 6) = 76.89$, $P < 0.001$) (Table 1). Similarly, in fluoxetine treated mice displacement curves were best fit to a one-site model for 5 out of 9 subjects (for pooled data of 5 subjects, $F(2, 6) = 0.84$, $P = 0.48$) and best fit by two-site model for 4 subjects (for pooled data of 4 subjects, $F(2, 6) = 70.36$, $P < 0.001$) (Table 1). The proportion of curves best fit to a one site model in drug treated mice was significantly different from vehicle ($P < 0.05$) in both drug treated groups. Neither citalopram nor fluoxetine treatment affected basal [^{3}H]CGP-39653 binding (Table 1).

4. Discussion

The present study demonstrates that chronic treatment with structurally dissimilar selective serotonin reuptake inhibitors citalopram and fluoxetine eliminates high-affinity glycine-displaceable [^{3}H]CGP-39653 binding to cortical membranes in a significant proportion of subjects. Previous studies have demonstrated that chronic treatment with a wide variety of antidepressants (including electroconvulsive shock) reduces the potency of glycine to inhibit [^{3}H]5,7-dichlorokynurenic acid binding in both mouse and rat cortex (Nowak et al., 1993; Paul et al., 1993, 1994). In addition, chronic administration of a more limited range of antidepressants (including imipramine, electroconvulsive shock, pargyline and amitriptyline was found to reduce the proportion of high affinity glycine-displaceable [^{3}H]CGP-39653 binding sites in rodent cortex while citalopram eliminated this high affinity component (Nowak et al., 1993, 1996; Paul et al., 1993; Huang et al., 1997).

Because citalopram is one of a class of selective serotonin reuptake inhibitors, the objective of the present study was to determine if this action was unique to citalopram or shared by other members of this family. The demonstration that chronic administration of both citalopram and fluoxetine eliminates high-affinity, glycine-displaceable [^{3}H]CGP-39653 binding sites in 78 and 56% of mice, respectively, indicates this may be a property common to selective serotonin reuptake inhibitors. As demonstrated by Mugnaini et al. (1993) the high affinity component of the displacement of [^{3}H]CGP-39653 binding to the glutamate recognition site by glycine is a consequence of an allosteric coupling between glycine and glutamate sites of the NMDA receptor complex. Thus, it is likely that citalopram and fluoxetine disrupt this allosteric coupling between the glycine and glutamate recognition sites. Chronic treatment of mice with citalopram and imipramine produce significant changes in mRNA levels encoding NMDA receptor subunits. However, there were both qualitative and quantitative differences in the effects of these antidepressants on mRNA levels (Boyer et al., data not shown). These findings suggest that differences in [^{3}H]CGP 39653 binding to NMDA receptors produced by selective serotonin reuptake inhibitors (e.g. citalopram, fluoxetine) and other antidepressants (e.g. imipramine, electroconvulsive shock) could reflect changes in subunit expression, and by implication, in NMDA receptor subunit composition. While further studies are required, the present findings are consistent with the hypothesis that perturbation of glutamatergic pathways is requisite for an antidepressant action (Huang et al., 1997; Shader et al., 1997).

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