ELSEVIER

European Journal of Pharmacology 295 (1996) 75–85

ejp

Adaptation of cortical but not hippocampal NMDA receptors after chronic citalopram treatment

Gabriel Nowak [a,*], Yong Li [c], Ian A. Paul [a,b]

[a] *Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS 39216-4505, USA*
[b] *Department of Pharmacology, University of Mississippi Medical Center, Jackson, MS 39216-4505, USA*
[c] *Laboratory of Neuroscience, NIDDK, National Institutes of Health, Bethesda, MD 20892-0008, USA*

Received 12 June 1995; revised 29 August 1995; accepted 15 September 1995

Abstract

Chronic treatment with citalopram produced a 6.2-fold reduction in the proportion of high affinity glycine-displaceable [^{3}H]CGP-39653 binding sites and a 1.5-fold reduction in the potency of glycine to inhibit [^{3}H]5,7-dichlorokynurenic acid binding in mouse cortex but not in hippocampus. Chronic citalopram also increased the aspartate concentration by 110% in cortex and 33% in hippocampus, and increased the glycine/threonine concentration by 33% in hippocampus. These results support the hypotheses that: (1) the adaptation of strychnine-insensitive glycine recognition sites and the allosteric coupling of the glycine and glutamate recognition sites are independently regulated by chronic antidepressant treatment; (2) chronic antidepressant administration induces regionally selective adaptation of the NMDA receptor complex; and (3) antidepressant-induced adaptation of the NMDA receptor complex may be mediated by regionally selective changes in excitatory amino acid concentration.

Keywords: Chronic citalopram; NMDA receptor; Excitatory amino acid; Adaptation; Cortex; Hippocampus

1. Introduction

Although the therapeutic efficacy of antidepressant treatments in the treatment of major depressive disorders is well established, the mechanism by which antidepressants exert this effect remains elusive. One of the most robust clinical observations relevant to this mechanism is the fact that antidepressants must be continuously administered for 3–6 weeks in order to achieve therapeutic effects (Heninger and Charney, 1987; Hollister and Csernansky, 1990). It is clear that the pharmacokinetic features of these treatments cannot account for this delay since steady-state blood and CNS concentrations of these drugs that are within therapeutic range can be achieved within 1 week of treatment (Oswald et al., 1972). Thus, the pharmacodynamic effects of these treatments appear to be responsible for the therapeutic response to treatment (see Hollister and Csernansky, 1990; Maj et al., 1984). This pharmacodynamic response to antidepressant treatment has been described as 'neural adaptation' to encompass the many possible neurobiological responses to the continued presence of antidepressants which may be of relevance to the therapeutic response to these treatments.

Citalopram is an antidepressant with a high potency and selectivity in inhibiting serotonin reuptake, measured in vitro or in vivo (Hyttel, 1978; Pawlowski et al., 1985; Pawlowski and Nowak, 1987). In comparative clinical studies, the efficacy of citalopram was similar to that of amitriptyline, maprotiline, clomipramine, mianserin and imipramine, with a more rapid therapeutic onset than mianserin and a less rapid onset than clomipramine (Milne and Goa, 1991; Bech and Cialdella, 1992; Van Harten, 1993; Rosenberg et al., 1994). Citalopram, like most selective serotonin reuptake inhibitors, is better tolerated by patients than tricyclic antidepressants (Milne and Goa, 1991; Bech and Cialdella, 1992; Van Harten, 1993; Rosenberg et al., 1994). Like the prototypic tricyclic antidepressants, chronic but not acute treatment with citalopram produces D_1 receptor downregulation (Klimek and

* Corresponding author. Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, 2500 North State St., Box 127, Jackson, MS 39216-4505, USA. Tel.: (601) 984-5744; fax: (601) 984-5899; e-mail: nowak@fiona.umsmed.edu.

SSDI 0014-2999(95)00585-4

Nielsen, 1987) and α_1-adrenoceptor upregulation (Vetulani et al., 1984; Nowak and Przegalinski, 1988). However, unlike tricyclics, citalopram treatment is without effect on forebrain β-adrenoceptors (Hyttel et al., 1984; Vetulani et al., 1984; Ordway et al., 1991) and is inactive in the forced swim test (see Hyttel, 1982), a behavioral test predictive for antidepressant activity (Porsolt et al., 1977; Borsini and Meli, 1988). As with other clinically active antidepressant treatments, the therapeutic mechanism of action of citalopram is unknown. Moreover, the acute ability of selective serotonin reuptake inhibitors, such as citalopram, to inhibit the reuptake of serotonin cannot account for the 3–6 weeks delay in the onset of therapeutic response to treatment (Hyttel et al., 1984).

Glutamatergic neurons and the *N*-methyl-D-aspartate (NMDA) receptor have been implicated in both the behavioral (Trullas and Skolnick, 1990; Trullas et al., 1991; Skolnick et al., 1992; Maj et al., 1992a, b) and the adaptive neuronal response to antidepressant treatments (Nowak et al., 1993; Paul et al., 1993, 1994). Similar to the other ligand-gated ion channels, the 'NMDA receptor complex' is constituted as a heterooligomer (Meguro et al., 1992; Kutswada et al., 1992) with multiple, allosterically coupled recognition sites for glutamate, glycine, polyamines, magnesium, zinc and use-dependent channel blockers (reviewed in Williams et al., 1991; Carter, 1992). However, the NMDA receptor complex differs from other ligand-gated ion channels in several respects. For example, the binding of both an agonist (glutamate) to its transmitter recognition site and of glycine to a strychnine-insensitive receptor is required for channel activation (Kleckner and Dingledine, 1988; Carter, 1992).

Chronic, but not acute treatment with clinically effective antidepressants results in adaptation of the NMDA receptor complex. This adaptation is manifest as a reduction in potency of glycine to inhibit [^{3}H]5,7-dichlorokynurenic acid (DCKA) binding to strychnine-insensitive glycine receptor and a reduction in the proportion of high affinity glycine binding sites inhibiting the [^{3}H]CGP-39653 binding to glutamate site of the NMDA receptor (Nowak et al., 1993; Paul et al., 1993, 1994). However, chronic citalopram treatment produces only modest effects on the potency of glycine to inhibit of [^{3}H]5,7-DCKA binding ($\sim$ 1.8-fold) compared to the 2–4-fold reductions in the potency of glycine to inhibit [^{3}H]5,7-DCKA binding typically observed after chronic antidepressant treatments (Paul et al., 1994). In contrast, preliminary studies have suggested that chronic citalopram treatment results in the most robust reduction in the proportion of high affinity glycine-displaceable [^{3}H]CGP-39653 binding sites of all antidepressants tested to date. These data suggest that a precise, stoichiometric relationship between adaptation at the strychnine-insensitive glycine recognition site and the glutamate recognition site does not necessarily occur.

Moreover, the mechanism by which antidepressants induce these changes is not known. While some antidepressants directly interact with the NMDA receptor complex in vitro (reviewed in Paul et al., 1994), the affinity of these drugs for the NMDA receptor complex is quite low, indicating that this mechanism is unlikely to account for antidepressant-induced adaptation of the NMDA receptor complex. Since the primary means for inactivating simple amino acid neurotransmitters is probably reuptake (Debler and Lajtha, 1987), one explanation for these preliminary data is that chronic administration of citalopram alters excitatory amino acid neurotransmitter release or uptake and hence might result in alterations in forebrain levels of either glycine or glutamate. The aims of the present study were to test: (1) the hypothesis that the stoichiometric relationship between the adaptation of the glycine and glutamate recognition sites of the NMDA receptor complex is not uniform among antidepressant treatments; (2) whether adaptation of the NMDA receptor complex in response to chronic citalopram treatment is region specific and; (3) whether chronic treatment with citalopram results in regionally selective alterations in forebrain concentrations of excitatory amino acids.

We now report that chronic treatment with citalopram produced only a moderate reduction in the potency of glycine to inhibit [^{3}H]5,7-DCKA binding to strychnine-insensitive glycine receptors, while producing a nearly complete elimination of high-affinity glycine displacement of [^{3}H]CGP-39653 binding to NMDA receptors. Thus, there is no direct stoichiometric relationship between the adaptation of strychnine-insensitive glycine recognition sites and the ability of glycine to displace antagonist binding to the glutamate recognition site of the NMDA receptor complex. In addition, the effects of citalopram treatment on both sites of the NMDA receptor complex were restricted to cortex, supporting the hypothesis that chronic antidepressant administration induces regionally selective adaptation of the NMDA receptor complex. Moreover, regionally specific alterations in forebrain levels of aspartate, but not glycine/threonine appear to parallel citalopram-induced adaptation of the NMDA receptor complex. These data are consistent with the hypothesis that adaptation of cortical glutamatergic neurons and the NMDA receptor complex is involved in the action of antidepressants.

2. Materials and methods

2.1. Animals

Male NIH Swiss-Webster mice (20–25 g, HSD) were housed in groups of 8–10 under a 12 h light/dark cycle

(lights on at 07:00 h) with free access to food and water until 3 h prior to killing. All procedures were conducted according to University of Mississippi Medical Center Animal Care and Use Committee guidelines. Citalopram (20 mg/kg i.p. – Lundbeck A/S – Copenhagen, Denmark) was administered once daily for 14 days. Animals were killed by decapitation 24 h after the last treatment, the brains were removed, neocortices (part of cortex localized above the rhinal sulcus) and hippocampi dissected, and placed on aluminum foil over solid CO_2. Tissues were stored at −80°C until assayed (2–6 weeks).

2.2. Radioligand binding assays

Radioligand binding assays were conducted essentially as previously described (Baron et al., 1991; Sills et al., 1991; Nowak et al., 1993). Initially, tissues were thawed in 50 volumes ice-cold 5 mM Hepes/4.5 mM Tris (HTS) buffer (pH 7.4) prepared using ultrapurified water exposed to UV light and passed through a final 0.22 μm filter (Solution 2001BU, Solution Consultants, Jasper, GA). This ultrapurified water preparation has previously been demonstrated to reduce total organic carbon to < 10–50 ppb (Solution Consultants) and amino acid concentrations to < 1 nM (R. Trullas, personal communication). Thawed tissues were then homogenized with a Brinkmann Polytron (setting 6, 20–30 s) and centrifuged at 20000 × *g* for 20 min.

2.2.1. [^{3}H]CGP-39653 binding assays

Tissue pellets were reconstituted in 50 volumes of HTS buffer containing 10 mM EDTA and centrifuged at 20000 × *g* for 20 min. The resulting tissue pellet was resuspended in 50 volumes of HTS buffer and stored at −80°C for at least 3 days. On the day of the assay, the frozen aliquot was thawed and washed 3 times with 50 volumes HTS. Assays consisted of membrane suspension (0.1–0.2 mg of protein), 10 nM [^{3}H]CGP-39653 (46 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. Sixteen 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 presoaked in 0.03% polyethyleneimine and washed twice with 5 ml of ice-cold HTS buffer. The filters were placed in 0.5 ml of Microscint-20 scintillation cocktail and the radioactivity measured in a Packard TopCount microplate scintillation counter.

2.2.2. [^{3}H]5,7-DCKA binding assays

Tissue pellets were washed twice in HTS containing 1 mM EDTA. The resulting tissue pellet was reconstituted in 5–10 volumes of HTS and stored at −80°C for at least 2 days prior to assay. On the day of the assay the frozen aliquot was washed once. The final tissue suspension was prepared in 50 mM Hepes-KOH buffer (pH 8.0). Assays consisted of membrane suspension (0.1–0.2 mg of protein), 20 nM [^{3}H]5,7-DCKA (15.8 Ci/mmol; DuPont-NEN) and buffer and/or drugs to a final volume of 1 ml. Nonspecific binding was defined in the presence of 100 μM of glycine. Eleven concentrations (10^{-4}–10^{-9} M) of glycine were used to construct inhibition curves. Incubation was terminated in a refrigerated centrifuge (15 min at 20000 × *g*). After aspirating the supernatant, pellets were superficially washed once with 1 ml Hepes-KOH buffer, dissolved in tissue solubilizer (Solvable, NEN-Research Products), and neutralized with glacial acetic acid. Scintillation cocktail (4 ml – Cytoscint, ICN Biomedicals, Irvine, CA) was added to this mixture and the radioactivity measured in a Beckman LS 3801 liquid scintillation counter.

All assays were performed in duplicate. Protein content was determined using Pierce (Rockford, IL) Protein Assay kits using the bicinchoninic acid method (Smith et al., 1985).

2.2.3. Amino acid analysis

Concentrations of amino acids were measured by high-performance liquid chromatography with electrochemical detection as previously described (Donzanti and Yamamoto, 1988). Tissue samples were sonicated in ice-cold $HCLO_4$ containing 10 μg/ml homoserine as an internal standard and were centrifuged at 13000 × *g* for 5 min at 4°C. The supernatant was collected and filtered through a 0.2 μm nylon syringe (Millipore, MA). Protein content of the pellet was determined as described above. The supernatant was analyzed using a Waters Maxima 820 analytical work station using coulometric electrochemical detection equipped with a dual electrode high sensitivity analytical cell (ESA Model 5100A, at a working potential of −0.03 and Model 5011 at a working potential of +0.65 V). The column was packed with Adsorbosphere C18 (100 × 4.6 mm – Alltech). The mobile phase was 0.1 M Na_2HPO_4, 0.13 mM Na_2EDTA and 33% methanol (pH 5.92) at a flow rate of 1.2 ml/min and pressure of 3.88 p.s.i. Concentrations of amino acids were determined by a comparison of peak area ratios of the samples with those of standards and are reported in ng of substance per μg of protein.

2.2.4. Data analysis and statistics

Data were analyzed using iterative curve fitting routines (GraphPAD/InPlot Ver. 4.04 or GraphPAD Prism Ver. 1.01). Group differences were assessed using analysis of variance (ANOVA) followed by Dunnett's post-hoc comparisons (Genstat 3.0, Research Triangle Park, NC) or repeated measures ANOVA

followed by Bonferroni-corrected univariate F-test (SYSTAT 5.0, Evanston, IL) as appropriate. Data were deemed significant when $P < 0.05$.

3. Results

3.1. Glycine displacement of [^{3}H]CGP-39653 binding

In both citalopram- and saline-treated mice, glycine displaced [^{3}H]CGP-39653 binding to cortical homogenates over a concentration range from 10^{-9} to 10^{-2} M (Fig. 1A). In controls, the single-site model for glycine displacement of [^{3}H]CGP-39653 possessed a very shallow slope (for pooled data, $n_H = 0.21$) and the data were significantly better fit for all subjects using a two-site model (for pooled data $F(1,75) = 5.10$, $P = 0.027$). However, in citalopram-treated mice, glycine displacement of [^{3}H]CGP-39653 possessed a slope that did not differ from unity (for pooled data, $n_H = 0.75$) and was best fit by one-site model for five of six subjects (for pooled data, $F(1,76) = 3.06$, $P = 0.084$). As a consequence, chronic treatment with citalopram reduced the proportion of high affinity glycine-dis-

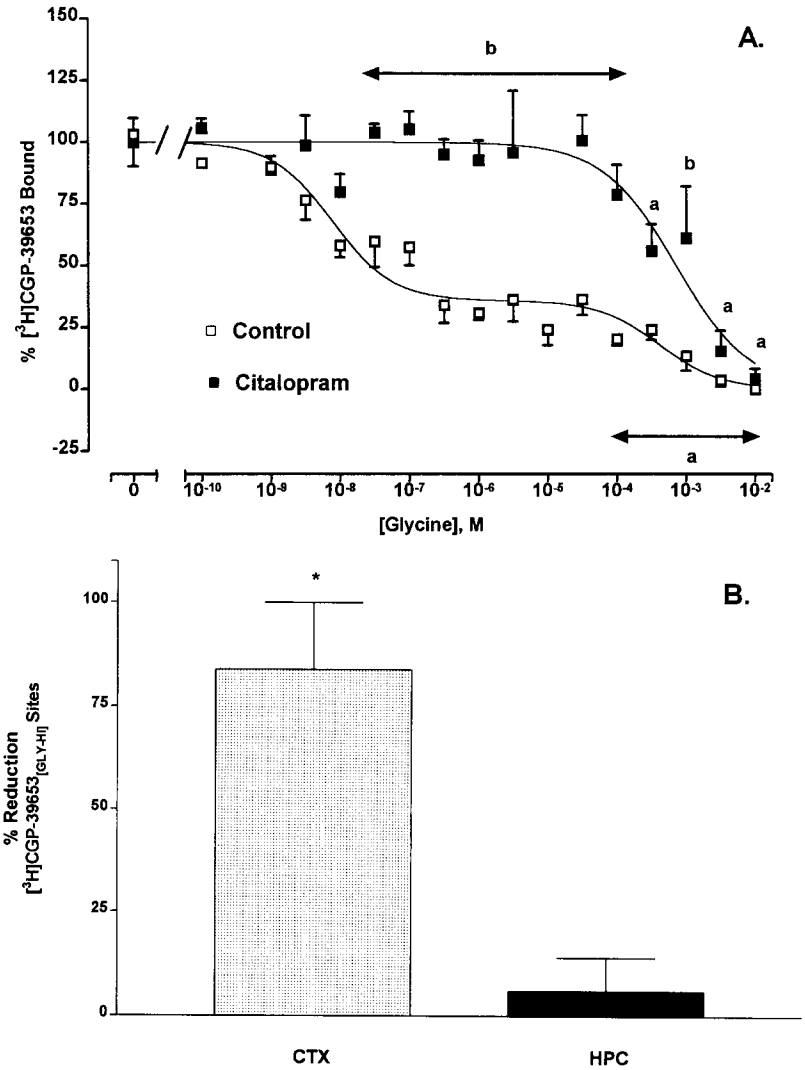

Fig. 1. Effect of chronic treatment with citalopram on glycine inhibition of [^{3}H]CGP-39653 binding. (A) Glycine inhibition [^{3}H]CGP-39653 binding to cortical membranes. Data are expressed as percent specific binding. Untransformed values are presented in Table 1. Data for saline-treated mice were best fit to a two-site model [$F(1,75) = 5.10$, $P = 0.027$] and to a one-site model for citalopram-treated mice [$F(1,76) = 3.06$, $P = 0.084$]. Two-factor (citalopram treatment by glycine concentration) ANOVA revealed main effects of citalopram [$F(1,123) = 134.6$, $P < 0.001$], glycine concentration [$F(14,123) = 22.1$, $P < 0.01$] and an interaction between citalopram treatment and glycine concentration [$F(14,123) = 3.48$, $P < 0.01$]. [a] $P < 0.05$ vs. 10^{-10} M glycine, [b] $P < 0.05$ vs. saline-control (Bonferoni-corrected univariate F-test). (B) Effect of chronic treatment with citalopram on glycine inhibition of [^{3}H]CGP-39653 binding in cortex (CTX) and hippocampus (HPC). Data are expressed as the percent reduction in the apparent density of high-affinity, glycine-displaceable [^{3}H]CGP-39653 binding sites and represent the means ± S.E.M. of four to six animals/group. * $P < 0.05$ vs. saline treatment (Dunnett's post hoc comparison).

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

Treatment	Specific binding (fmol/mg protein)	Site 1 IC_{50} (nM)	% Site 1	Site 2 IC_{50} (μM)	2 Site/ 1 site
Cortex					
Control	389 ± 40	44.0 ± 26.0	68 ± 4	2310 ± 1707	6/0
Citalopram	317 ± 32	0.13 ± 0.13	11 ± 11 [a]	1058 ± 386	1/5 [b]
Hippocampus					
Control	624 ± 76	165 ± 105	52 ± 7	1139 ± 492	4/0
Citalopram	766 ± 47	116 ± 66	44 ± 4	2492 ± 1368	5/0

Animals were treated with saline or citalopram (20 mg/kg i.p.) for 14 days. 24 h after the last administration, the animals were killed and [^{3}H]CGP-39653 binding was assayed as described in Methods. A one-site model was assumed unless the sum of squares of the model was significantly reduced by employing a two-site model (F-test, GraphPAD Prism 1.01). Data represent the means ± S.E.M. of four to six mice/group. [a] $P < 0.05$ vs. control (Dunnett's t-test), [b] $P < 0.01$ vs. control (Fisher's Exact Test – 2 site/1 site).

placeable [^{3}H]CGP-39653 binding sites in cortex by 89% (Table 1; Fig. 1A). In contrast, citalopram treatment did not affect either specific [^{3}H]CGP-39653 binding or the potency of glycine to inhibit [^{3}H]CGP-39653 binding at either the high or low affinity sites in cortical homogenates (Table 1; Fig. 1A).

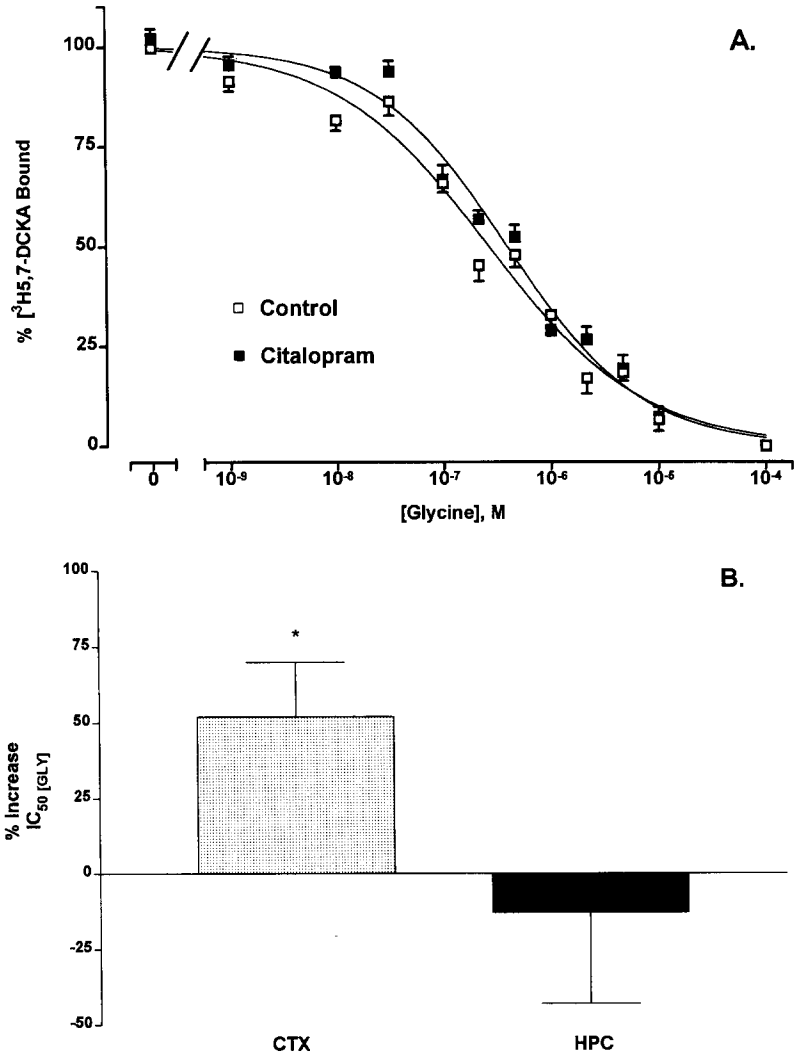

Fig. 2. Effect of chronic treatment with citalopram on glycine inhibition of [^{3}H]5,7-DCKA binding. (A) Glycine inhibition [^{3}H]5,7-DCKA binding to cortical membranes. Data are expressed as percentages specific binding. Untransformed values are presented in Table 1. (B) Effect of chronic treatment with citalopram on glycine inhibition of [^{3}H]5,7-DCKA binding in cortex (CTX) and hippocampus (HPC). Data are expressed as the percentages increase in IC_{50} vs. saline and represent the means ± S.E.M. of six to nine animals/group). * $P < 0.05$ vs. saline (Dunnett's t-test).

Glycine also displaced [^{3}H]CGP-39653 binding to hippocampal homogenates over a concentration range from 10^{-9} to 10^{-2} M (Fig. 1B). However, in contrast to the results obtained with cortical homogenates, in both control and citalopram-treated mice, the single-site model for glycine displacement of [^{3}H]CGP-39653 possessed a very shallow slope (for pooled data, $n_H = 0.28$ and $n_H = 0.19$, for saline- and citalopram-treated, respectively) and the data were significantly better fit for all subjects using a two-site model (pooled data $F(1,43) = 4.20$, $P = 0.047$ and $F(1,65) = 12.50$, $P = 0.0008$, for saline- and citalopram-treated, respectively). Likewise, citalopram treatment did not affect either specific [^{3}H]CGP-39653 binding or the potency of glycine to inhibit [^{3}H]CGP-39653 binding at either the high or low affinity sites in hippocampal homogenates (Table 1; Fig. 1B).

3.2. Glycine displacement of [^{3}H]5,7-DCKA binding

Chronic treatment with citalopram reduced the potency (increased the IC_{50} value) of glycine to inhibit [^{3}H]5,7-DCKA binding 1.5-fold in cortex, but not in hippocampus (Fig. 2A,B). This treatment did not affect specific [^{3}H]5,7-DCKA binding in either brain region (Table 2).

3.3. Amino acid concentrations

Chronic treatment with citalopram resulted in a 110 ± 29% increase in the concentration of aspartate in cortex but, did not significantly affect cortical concentrations of glutamate, serine, glutamine, glycine/threonine, taurine or alanine (Table 3). Similarly, chronic citalopram treatment resulted in a significant increase in the hippocampal concentration of asparatate (33 ± 10%) and glycine/threonine (33 ± 7%) without affecting hippocampal concentrations of other excitatory amino acids (Table 3).

Table 2
Effect of chronic treatment with citalopram on glycine inhibition of [^{3}H]5,7-DCKA binding to mouse cortex and hippocampus

Treatment	Specific binding (fmol/mg protein)	IC_{50} (nM)	n_H
Cortex			
Control	910 ± 54	275 ± 32	0.64 ± 0.06
Citalopram	861 ± 57	417 ± 50 [a]	0.71 ± 0.07
Hippocampus			
Control	520 ± 84	495 ± 146	0.82 ± 0.21
Citalopram	460 ± 71	433 ± 149	0.67 ± 0.08

Animals were treated with saline or citalopram (20 mg/kg i.p.) for 14 days. 24 h after the last administration, the animals were killed and [^{3}H]5,7-DCKA binding was assayed as described in Methods. A one-site model was assumed unless the sum of squares of the model was significantly reduced by employing a two-site model (F-test, GraphPAD Prism 1.01). Data represent the means ± S.E.M. of six to nine mice/group. [a] $P < 0.05$ vs. control (Dunnett's t-test).

Table 3
Effect of chronic treatment with citalopram on excitatory amino acid concentrations in mouse cortex and hippocampus

Amino acid	Cortex		Hippocampus	
	Control	Citalopram	Control	Citalopram
Aspartate	14.4 ± 0.9	30.2 ± 4.2 [a]	10.1 ± 0.4	13.4 ± 1.0 [a]
Glutamate	32.0 ± 2.5	44.4 ± 6.3	64.4 ± 3.6	72.5 ± 2.6
Serine	2.4 ± 0.4	3.2 ± 0.6	5.7 ± 0.2	6.2 ± 0.3
Glutamine	18.5 ± 1.7	24.5 ± 3.9	13.3 ± 0.4	13.7 ± 0.7
Glycine/threonine	1.7 ± 0.3	1.7 ± 0.4	3.0 ± 0.1	4.0 ± 0.2 [a]
Taurine	73.1 ± 8.0	107.2 ± 11.8	60.1 ± 2.2	62.2 ± 2.3
Alanine	1.0 ± 0.1	1.5 ± 0.2	1.9 ± 0.1	2.3 ± 0.2

Animals were treated with saline or citalopram (20 mg/kg i.p.) for 14 days. 24 h after the last administration, the animals were killed and excitatory amino acid concentrations were assayed as described in Methods. Data are expressed in ng/μg protein and represent the means ± S.E.M. of four mice/group. [a] $P < 0.05$ vs. control.

4. Discussion

Antidepressant drugs possess high to moderate affinity for the NMDA receptor complex in radioreceptor binding in vitro and in functional studies ex vivo (Reynolds and Miller, 1988; Sills and Loo, 1989; Kitamura et al., 1991; Cai and McCaslin, 1992; McCaslin et al., 1992; Mjellem et al., 1993). In addition, chronic antidepressant treatment in vivo affects excitatory amino acid concentrations in both mouse brain (Kitamura et al., 1991) and in cerebrospinal fluid (CSF) samples from depressed patients (Pangalos et al., 1992).

Functional antagonists at both the glycine and glutamate recognition sites of the NMDA receptor complex are active in preclinical rodent behavioral screening procedures sensitive to antidepressants (Trullas and Skolnick, 1990; Trullas et al., 1991; Skolnick et al., 1992; Maj et al., 1992a, b). Likewise, chronic administration of NMDA receptor antagonists reverses chronic mild stress-induced reductions in sucrose drinking in rats, a rodent model of depression (Papp and Moryl, 1993, 1994). Moreover, chronic but not acute treatment with NMDA receptor antagonists results in a reduction in the density of β-adrenoceptors (Paul et al., 1992; Klimek and Papp, 1994).

Our previous studies have demonstrated that chronic treatment with antidepressants from all of the principal pharmacological groups as well as electroconvulsive shock reduce the potency of glycine to inhibit [^{3}H]5,7-DCKA binding in both mouse and rat cortex (Nowak et al., 1993; Paul et al., 1993, 1994). In addition, we have demonstrated that chronic administration of imipramine or electroconvulsive shock reduces the proportion of high affinity glycine-displaceable [^{3}H]CGP-

39653 binding sites in rodent cortex (Nowak et al., 1993; Paul et al., 1993). In both measures, imipramine-induced adaptation of the NMDA receptor complex was regionally selective, being observed in cerebral cortex, but not in hippocampus, striatum or basal forebrain (Nowak et al., 1993). In preliminary studies we found that chronic treatment with some other antidepressants (amitriptyline, pargyline and citalopram) also reduces the proportion of high affinity glycine-displaceable [^{3}H]CGP-39653 binding sites in mouse cortex (Layer et al., 1995).

The present studies confirm and extend these preliminary results, demonstrating that chronic administration of citalopram results in robust, regionally selective effects on the displacement of [^{3}H]CGP-39653 and [^{3}H]5,7-DCKA binding by glycine. Thus, chronic citalopram treatment resulted in reductions in the proportion of high-affinity, glycine-displaceable [^{3}H]CGP-39653 binding sites and in the potency of glycine to inhibit [^{3}H]5,7-DCKA binding in cortical, but not hippocampal membranes.

In addition, the present study demonstrates that the effect of chronic treatment with antidepressants does not necessarily result in stoichiometrically equivalent effects on both the glycine and glutamate recognition sites of the NMDA receptor complex. Thus, chronic administration of citalopram results in essentially complete elimination of the high-affinity component of glycine displacement of [^{3}H]CGP-39653 to the glutamate site while resulting in a modest ~1.5-fold increase in the IC_{50} of glycine to displace [^{3}H]5,7-DCKA binding to the glycine site. By comparison, the effect of chronic imipramine and electroconvulsive shock treatments, ranges between 2- to 4-fold for both the proportion of high-affinity glycine-displaceable [^{3}H]CGP-39653 binding sites and for the reduction in the potency of glycine to displace [^{3}H]5,7-DCKA binding (Nowak et al., 1993; Paul et al., 1993, 1994). Thus, it appears that antidepressants can regulate the glycine and glutamate recognition sites of the NMDA receptor complex independently of one another.

In earlier studies of the time-course and dose-response of antidepressant-induced adaptation of the NMDA receptor complex we have demonstrated that imipramine is significantly more efficaceous than citalopram (~330% vs. 200%, respectively) at reducing the potency of glycine to inhibit [^{3}H]5,7-DCKA binding to the glycine recognition site of the NMDA receptor complex (Paul et al., 1994). However, the minimum effective dose for these treatments to induce adaptation of this site is similar (Paul et al., 1994). In the present study, chronic administration of citalopram resulted in nearly complete elimination (~6.2-fold reduction) of the high affinity component of glycine-displaceable [^{3}H]CGP-39653 binding to the glutamate recognition site of the NMDA receptor complex. By contrast, previous studies with imipramine have resulted in a much less robust reduction in the proportion of high affinity, glycine-displaceable [^{3}H]CGP-39653 binding (~1.4-fold – Nowak et al., 1993). As recently demonstrated by Mugnaini et al. (1993) this 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. In contrast, Yoneda et al. (1993) have reported that the inhibition of [^{3}H]5,7-DCKA binding by glycine is consistent with a competitive mechanism. Taken together, these data indicate that imipramine treatment has a more significant impact on the strychnine-insenstive glycine recognition site of the NMDA receptor complex than on the allosteric coupling of the glycine and glutamate recognition sites. Conversely, citalopram robustly disrupts the allosteric coupling between the glycine and glutamate recognition sites while slightly affects the strychnine-insensitive glycine recognition site. While further studies will be required, these differences in the response of the NMDA receptor complex to antidepressant treatments, provide a potential mechanism to account for the nonuniform clinical response to antidepressant treatments.

Recently, Grimwood et al. (1993) have demonstrated that glutamate site antagonists appear to interact with the distinct glutamate recognition site of the NMDA receptor complex. These glutamate recognition site subtypes are diffentially sensitive to glutamate antagonists with a 5 or 7 carbons linking carboxyl-phosphonate moeities (so called 'C-5' and 'C-7' antagonists). Moreover, the modulation of radioligand binding to these subtypes by stychnine-insensitive glycine site ligands is distinct (Grimwood et al., 1993). Thus it could be argued that CGP-39653 (and other so called 'C-5' antagonists such as CGS-19755) may bind not only to the glutamate site of the NMDA receptor complex but also to the strychnine-insensitive glycine recognition site. This hypothesis would imply that the high affinity component of glycine displacement of [^{3}H]CGP-39653 from the glutamate binding site is in reality glycine displacement of [^{3}H]CGP-39653 binding to glycine sites. However, only agonists of the strychnine-insensitive glycine recognition site (glycine and D-serine) displace [^{3}H]CGP-39653 binding with both high and low affinity components. In contrast glycine site antagonists, such as 7-chlorokynurenic acid displace [^{3}H]-CGP-39653 with only a single low affinty component (Mugnaini et al., 1993). Moreover, the high affinity component of glycine inhibition of the [^{3}H]CGP-39653 binding is dose dependently reversed by 7-chlorokynurenic acid. These findings indicate that it is unlikely that high affinity glycine-displaceable [^{3}H]CGP-39653 binding is due to a competitive interaction be-

tween glycine and [^{3}H]CGP-39653 at the strychnine-insensitive glycine sites. In spite of the possible arguments that the antidepressant treatment induce alterations in spanning (or spatial arrangement) of these two sites (glutamate and glycine), we accept as more likely the hypothesis that [^{3}H]CGP-39653 binds to glutamate sites which are modulated by glycine site agonists and that this modulation is altered by chronic antidepressant treatment.

The present data also demonstrate that, like imipramine, citalopram induces adaptative changes in a regionally selective manner with effects being observed in cortex but not in hippocampus. It is noteworthy that in spite of the robust effect of citalopram on the proportion of high affinity glycine-displaceable [^{3}H]CGP-39653 binding sites in cortex, this effect is not present in hippocampus, supporting the notion that adaptation of the NMDA receptor complex is specific to cerebral cortex. Moreover, these data suggest that antidepressant-induced adaptation of the NMDA receptor involves a relatively discrete subpopulation of glutamatergic neurons with projections in or to the cortex but not hippocampus.

As discussed previously, the significance of the adaptation of the response of the NMDA receptor complex to glycine would be of little importance if strychnine-insensitive glycine receptors were saturated in vivo (Paul et al., 1993, 1994). The reported concentration of glycine in extracellular extracts and cerebrospinal fluid is high enough to saturate these receptors (reviewed in Kemp and Leeson, 1993). However, several lines of evidence indicate receptor saturation may not be obtained in situ (reviewed in Thomson, 1990). For example, glycine has been shown to potentiate the actions of NMDA in both neocortical slices ex vivo (Thomson, 1990) and thalamic, spinal cord and hippocampal neurons in vivo (Salt, 1989; Budai et al., 1992; Thiels et al., 1992). Moreover, administration of glycine, D-serine (a high affinity ligand at strychnine-insensitive glycine receptors) or D-cycloserine (a partial agonist at the strychnine-insensitive glycine receptors) elevates in vivo cerebellar cyclic GMP levels and this effect is antagonized by NMDA antagonists (Wood et al., 1989; Danysz et al., 1989; Rao et al., 1990) and enhances the excitability of the hippocampal dentate granule cells in vivo (Pitkanen et al., 1994). These findings, taken together with the presence of a high capacity, low affinity uptake system for glycine in the central nervous system (Trullas et al., 1991) indicate that the antidepressant-induced reductions in the potency of glycine for strychnine-insensitive glycine sites and in the ability of glycine to allosterically modulate glutamate sites could have a profound impact on the operation of the NMDA receptor complex.

In view of the essential role of glycine in the operation of NMDA receptor-coupled cation channels (Kleckner and Dingledine, 1988; Carter, 1992), the present and the previous (Nowak et al., 1993; Paul et al., 1993, 1994) findings indicate that chronic antidepressant treatment impairs operation of NMDA receptor-coupled cation channels selectively in the cerebral cortex. Thus, it is of interest to note that in vivo computer tomography and magnetic resonance imaging studies have reported frontal cortical dysfunction in both unipolar and bipolar depression in humans (Robinson and Szetela, 1981; Starkstein et al., 1987, 1988, 1989; Sinyor et al., 1986; Baxter et al., 1989; Coffey et al., 1993). The present data together with our previous results are thus in good accord with these studies in humans and support the hypothesis that involvement of a cortical glutamate-NMDA system represents a final common pathway of antidepressant activity and potentially of the pathophysiology of human depression.

The mechanisms by which antidepressant treatments such as citalopram effect adaptative changes in ligand binding to the NMDA receptor complex are unknown. Reynolds and Miller (1988) and Kitamura et al. (1991) have reported that certain tri- and tetracyclic antidepressants inhibit [^{3}H]dizocilpine binding to rodent brain. Likewise, we have found citalopram to possess moderate affinity to displace [^{3}H]dizocilpine ($IC_{50} = 37 \pm 3$ μM – Nowak et al., unpublished data) which is comparable to that of imipramine ($IC_{50} = 16 \pm 1$ μM – Nowak et al., unpublished data) and other antidepressants in this system (Reynolds and Miller, 1988; Kitamura et al., 1991). While it is possible that the adaptation produced by antidepressants such as citalopram at the dose employed in the present study might be achieved by a direct interaction with the NMDA receptor complex, the relatively low affinities of other antidepressants (e.g. mianserin, $IC_{50} \sim 300$ μM) makes this an unlikely mechanism to account for the adaptation of the NMDA receptor complex across treatments. Moreover, this explanation cannot account for the distinct differences in the response of the NMDA receptor complex to antidepressants such as imipramine and citalopram which possess very similar potency to displace [^{3}H]dizocilpine.

In the present study, we examined the possibility that an antidepressant which induces a robust 'decoupling' of the glycine and glutamate sites of the NMDA receptor complex might also induce changes in forebrain excitatory amino acid levels. The observed doubling of cortical aspartate levels following chronic citalopram treatment is robust ($\sim 110\%$) and could potentialy be involved in the mechanism of the disruption in the coupling between glycine and glutamate sites in cortex via a hyperstimulation of the glutamate recognition site of the NMDA receptor complex. This hypothesis is supported by the fact that chronic citalopram treatment did not result in decoupling of the

glycine-glutamate sites in hippocampus where aspartate concentrations were more modestly affected than in cortex. Conversely, the concentration of glycine/threonine is increased only in the hippocampus where no adaptation of the NMDA receptor complex was evident. Thus, alterations in forebrain concentrations of these amino acids do not appear to be directly related to the the adaptation of the NMDA receptor complex, since this effect is observed only in cortex.

Kitamura et al. (1991) detected 8–15% reductions in excitatory amino acid concentrations in mouse hippocampus following chronic treatment with desipramine. The differences between the present study and that of Kitamura et al. (1991) may be related to differences in the mechanism of action of citalopram and desipramine. Alternatively, it is possible that citalopram produces transient changes in the excitatory amino acid concentrations during chronic treatment, which participate in inducing receptor adaptation, but are not detectable 24 h after the last treatment. Additional studies will be needed to resolve this issue. Nevertheless, the present data represent the first demonstration of alterations in forebrain concentrations of excitatory amino acids following chronic treatment with an antidepressant drawn from the class of 'selective serotonin uptake inhibitors'. In addition, these data suggest that selective alterations in presynaptic concentrations of excitatory amino acids may play a role in the adaptation of the NMDA receptor complex, induced by chronic antidepressant treatment. Thus, these data indicate that both pre- and postsynaptic elements of glutamatergic pathways may be affected by chronic antidepressant administration.

In summary, the present data are consistent with the hypothesis that adaptation of glutamatergic neurons and the NMDA receptor complex is involved in the action of antidepressants. In addition, these data lend further support to the hypothesis that these effects are specific within forebrain structures. Moreover, the present findings demonstrate that the adaptation of strychnine-insensitive glycine recognition sites and the allosteric coupling of the glycine and glutamate recognition sites of the NMDA receptor complex are independently regulated by chronic antidepressant treatment.

Acknowledgements

Authors thank H. Lundbeck A/S (Copenhagen, Denmark) for generous gift of citalopram. G.N. is on leave from the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

References

Baron, B.M., B.W. Siegel, A.L. Sloan, B.L. Harrison, M.G. Palfreyman and S.D. Hurt, 1991, [^{3}H]5,7-Dichlorokynurenic acid, a novel radioligand labels NMDA receptor, Eur. J. Pharmacol. 206, 149.

Baxter, L.R., J.M. Schwartz, M.E. Phelps, J.C. Mazziotta, B.M. Guze, C.E. Selin, R.H. Gerner and R.M. Sumida, 1989, Reduction of prefrontal cortex glucose metabolism common to three types of depression, Arch. Gen. Psychiatry 46, 243.

Bech, P. and P. Cialdella, 1992, Citalopram in depression-meta-analysis of intended and unintended effects, Int. Clin. Psychopharmacol. 6 Suppl 5, 45.

Borsini, F. and A. Meli, 1988, Is the forced swim test a suitable model for revealing antidepressant activity?, Psychopharmacology 94, 147.

Budai, D., G.L. Wilcox and A.A. Larson, 1992, Enhancement of NMDA-evoked neuronal activity by glycine in the rat spinal cord in vivo, Neurosci. Lett. 135, 265.

Cai, Z. and P.P. McCaslin, 1992, Amitriptyline, desipramine, cyproheptadine and carbamazepine, in concentrations used therapeutically, reduce kainate- and *N*-methyl-D-aspartate-induced intracellular Ca_{2+} levels in neuronal culture, Eur. J. Pharmacol. 219, 53.

Carter, A.J., 1992, Glycine antagonists: regulation of the NMDA receptor-channel complex by the strychnine-insensitive glycine site, Drugs of the Future 17, 595.

Coffey, C.E., W.E. Wilkinson, R.D. Werner, I.A. Parashos, W.T. Djang, M.C. Webb, G.S. Figiel and C.E. Spritzer, 1993, Quantitative cerebral anatomy in depression, Arch. Gen. Psychiatry 50, 7.

Danysz, W., J.T. Wroblewski, G. Brooker and E. Costa, 1989, Modulation of glutamate receptors by phencyclidine and glycine in the rat cerebellum: cGMP increases in vivo, Brain Res. 479, 270.

Debler, E.A. and A. Lajtha, 1987, High-affinity transport of gamma-aminobutyric acid, glycine, taurine, L-aspartic acid, and L-glutamic acid in synaptosomal (P_2) tissue: a kinetic and substrate specificity analysis, J. Neurochem. 48, 1851.

Donzanti, B.A. and B.K. Yamamoto, 1988, An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates, Life Sci. 43, 913.

Grimwood, S., G.J.C. Wilde and A.C. Foster, 1993, Interactions between the glutamate and glycine recognition sites of the *N*-methyl-D-asoartate receptor from rat brain, as revealed from radioligand binding studies, J. Neurochem. 60, 1729.

Heninger, G. and Charney, D., 1987, Mechanism of action of antidepressant treatments: implications for the etiology and treatment of depressive disorders, in: Psychopharmacology: The Third Generation of Progress, ed. H. Meltzer (Raven Press, New York) p. 535.

Hollister, L.E. and J.G. Csernansky (eds.), 1990, Clinical Pharmacology of Psychotherapeutic Drugs, 3rd edn. (Churchill Livingston, New York).

Hyttel, J., 1978, Effect of a specific 5-HT uptake inhibitor, citalopram (Lu 10-171), on ^{3}H-5-HT uptake in rat brain synaptosomes in vitro, Psychopharmacology 60, 13.

Hyttel, J., 1982, Citalopram – pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 6, 277.

Hyttel, J., K. Fredricson-Overo and J. Arnt, 1984, Biochemical effects and drug levels in rats after long-term treatment with the specific 5-HT uptake inhibitor, citalopram, Psychopharmacology 83, 20.

Kemp, J.A. and P.D. Leeson, 1993, The glycine site of the NMDA receptor – five years on, Trends Pharmacol. Sci. 14, 20.

Kitamura, Y., X.-H. Zhao, M. Takei, O. Yonemitsu and Y. Nomura, 1991, Effects of antidepressants on the glutamatergic system in mouse brain, Neurochem. Int. 3, 247.

Kleckner, N.W. and R. Dingledine, 1988, requirement for glycine in activation of NMDA-receptors expressed in Xenopus, Science 241, 835.

Klimek, V. and M. Nielsen, 1987, Chronic treatment with antidepressants decreases the number of [^{3}H]SCH 23390 binding sites in rat striatum and limbic system, Eur. J. Pharmacol. 139, 163.

Klimek, V. and M. Papp, 1994, The effect of MK-801 and imipramine on beta-adrenergic and 5-HT_2 receptors in the chronic mild stress model of depression in rats, Pol. J. Pharmacol. 46, 67.

Kutswada, T., N. Kashiwabuchi, S. Mori, E. Kushiya, K. Araki, H. Meguro, H. Masaki, T. Kumanishi, M. Arakawa and M. Mishina, 1992, Molecular diversity of the NMDA receptor channel, Nature 358, 36.

Layer, R.T., P. Popik, G. Nowak, I.A. Paul, R. Trullas, and P. Skolnick, 1995, A unified theory of antidepressnt action: evidence for adaptation of the *N*-methyl-D-aspartate (NMDA) receptor complex following chronic antidepressant treatment, in: Ion Channel Pharmacology, eds. V. Cena and B. Soria (Oxford University Press, London) (in press).

Maj, J., E. Przegalinski and E. Mogilnicka, 1984, Hypotheses concerning the mechanism of action of antidepressant drugs, Rev. Physiol. Biochem. Pharmacol. 100, 1.

Maj, J., Z. Rogóz, G. Skuza and H. Sowinska, 1992a, The effects of MK-801 and antidepressant drugs in the forced swimming test in rats, Eur. J. Neuropsychopharmacol. 2, 37.

Maj, J., Z. Rogoz, G. Skuza and H. Sowinska, 1992b, The effect of CGP 37849 and CGP 39551, competitive NMDA antagonists, in the forced swimming test, Pol. J. Pharmacol. Pharm. 44, 337.

McCaslin, P.P., X.Z. Yu, I.K. Ho and T.G. Smith, 1992, Amitriptyline prevents *N*-methyl-D-aspartate (NMDA)-induced toxicity, does not prevent NMDA-induced elevation of extracellular glutamate, but auguments kainate-induced elevation of glutamate, J. Neurochem. 59, 401.

Meguro, H., H. Mori, K. Araki, E. Kushiya, T. Kutsuwada, M. Yamazaki, T. Kumanishi, M. Arakawa, K. Sakimura and M. Mishina, 1992, Functional characterization of a heteromeric NMDA receptor channel from cloned cDNAs, Nature 357, 70.

Milne, R.J. and K.L. Goa, 1991, Citalopram: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in depressive illness, Drugs 41, 450.

Mjellem, N., A. Lund and K. Hole, 1993, Reduction of NMDA-induced behaviour after acute and chronic administration of desipramine in mice, Neuropharmacol. 32, 591.

Mugnaini, M., A. Giberti, E. Ratti and F.T.M. Van Amsterdam, 1993, Allosteric modulation of [^{3}H]CGP-39653 binding by glycine in rat brain, J. Neurochem. 61, 1492.

Nowak, G. and E. Przegalinski, 1988, Effect of repeated treatment with antidepressant drugs and electroconvulsive shock (ECS) on ^{3}H-prazosin binding to diffent brain structures, J. Neural Transm. 71, 57.

Nowak, G., R. Trullas, R.T. Layer, P. Skolnick and I.A. Paul, 1993, Adaptive changes in the *N*-methyl-D-aspartate receptor complex after chronic treatment with imipramine and 1-aminocyclopropanecarboxylic acid, J. Pharmacol. Exp. Ther. 265, 1380.

Ordway, G.A., C. Gambarana, S.M. Tejani-Butt, P. Areso, M. Hauptmann and A. Frazer, 1991, Preferential reduction of binding of ^{125}I-iodopindolol to beta-1 adrenoceptors in the amygdala of rat after antidepressant treatments, J. Pharmacol. Exp. Ther. 257, 681.

Oswald, I., V. Brezinowa and D.L.F. Dunleavy, 1972, On the slowness of action of tricyclic antidepressant drugs, Br. J. Psychiatry 120, 673.

Pangalos, M.N., A.L. Malizia, P.T. Francis, S.L. Lowe, P.H. Bertolucci, A.W. Procter, P.K. Bridges, J.R. Bartlett and D.M. Bowen, 1992, Effect of psychotropic drugs on excitatory amino acids in patients undergoing psychosurgery for depression, Br. J. Psychiatry 160, 638.

Papp, M. and E. Moryl, 1993, Similar effect of chronic treatment with imipramine and the NMDA antagonists CGP 37849 and MK-801 in a chronic mild stress model of depression in rats, Eur. J. Neuropsychopharmacol. 3, 348.

Papp, M. and E. Moryl, 1994, Antidepressant activity of non-competitive and competitive NMDA antagonists in a chronic mild stress model of depression, Eur. J. Pharmacol. 263, 1.

Paul, I.A., R. Trullas, P. Skolnick and G. Nowak, 1992, Down-regulation of cortical beta-adrenoceptors by chronic treatment with functional NMDA antagonists, Psychopharmacology (Berlin) 106, 285.

Paul, I.A., R.T. Layer, P. Skolnick and G. Nowak, 1993, Adaptation of the NMDA receptor in rat cortex following chronic electroconvulsive shock or imipramine, Eur. J. Pharmacol. Mol. Pharmacol. Sect. 247, 305.

Paul, I.A., G. Nowak, R.T. Layer, P. Popik and P. Skolnick 1994, Adaptation of the *N*-methyl-D-aspartate receptor complex following chronic antidepressant treatments, J. Pharmacol. Exp. Ther. 269, 95.

Pawlowski, L., G. Nowak, Z. Gorka, and H. Mazela, 1985, Ro 11-2465 (cyan-imipramine), citalopram and their *N*-desmethyl metabolites: effects on the uptake of 5-HT and NA in vivo and related pharmacological activities, Psychopharmacology 86, 156.

Pawlowski, L. and G. Nowak, 1987, Biochemical and pharmacological tests for the prediction of ability of monoamine uptake blockers to inhibit the uptake of noradrenaline in vivo: the effects of desipramine, maprotiline, femoxetine and citalopram, J. Pharm. Pharmacol. 39, 1003.

Pitkanen, M., J. Sirvio, H. Lahtinen, E. Koivisto and P. Riekkinen, 1994, d-Cycloserine, a partial agonist at the glycine site, enhances the excitability of dentate granule cells in vivo in rats, Eur. J. Pharmacol. 253, 125.

Porsolt, R.D., A. Bertin and M. Jalfre, 1977, Behavioral despair in mice: a primary screening test for antidepressants, Arch. Int. Pharmacodyn. Ther. 229, 327.

Rao, T., J. Cler, M. Emmett, S. Mick, S. Iyengar and P. Wood, 1990, Glycine,glycinamide,and D-serine act as positive modulators of signal tranduction at the *N*-methyl-D-aspartate (NMDA) receptor in vivo: differential effects on mouse cerebellar cyclic guanosine monophosphate levels, Neuropharmacology 29, 1075.

Reynolds, I.J. and R.J. Miller, 1988, multiple sites for the regulation of the NMDA receptor, Mol. Pharmacol. 33, 581.

Robinson, S.L. and B. Szetela, 1981, Mood changes following left hemispheric brain injury, Ann. Neurol. 9, 447.

Rosenberg, C.D., N. Damsbo, E. Fuglum, L.V. Jacobsen and S. Horsgard, 1994, Citalopram and imipramine in the treatment of depressive patients in general practice. A Nordic multicentre clinical study, Int. Clin. Psychopharmacol. 9 (Suppl.1), 41.

Salt, T.E., 1989, Modulation of the NMDA receptor mediated responses by glycine and D-serine in the rat thalamus in vivo, Brain Res. 481, 403.

Sills, M.A. and P.S. Loo, 1989, Tricyclic antidepressants and dextromethorphan bind with higher affinity to the phencyclidine receptor in the absence of magnesium and L-glutamate, Mol. Pharmacol. 36, 160.

Sills, M.G., G. Fagg, M. Pozza, C. Angst, D. Brundish, S.D. Hurt, E.J. Wilusz and M. Williams, 1991, [^{3}H]CGP 39653: a new *N*-methyl-D-aspartate antagonist radioligand with low nanomolar affinity in rat brain, Eur. J. Pharmacol. 192, 19.

Sinyor, D., P. Jacques, D.G. Kaloupek, R. Becker, M. Goldenberg and H.M. Coopersmith, 1986, Post-stroke depression and lesion location: An attempted replication, Brain 109, 537.

Skolnick, P., R. Miller, A. Young, K. Boje and R. Trullas, 1992, Chronic treatment with 1-aminocyclopropanecarboxylic acid desensitizes behavioral responses to compounds acting at the *N*-methyl-D-aspartate receptor complex, Psychopharmacol. (Berlin) 107, 489.

Smith, P.K., R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.I. Gartner, M.D. Provezano, E.K. Fujimoto, N.M. Goeke, B.J. Olson and D.C. Klenk, 1985, Measurement of protein using bicinchoninic acid, Anal. Biochem. 150, 76.

Starkstein, S.F., R.G. Robinson and T.R. Price, 1987, Comparison of cortical and subcortical lesions in the production of post-stroke mood disorders, Brain 110, 1045.

Starkstein, S.F., R.G. Robinson and T.R. Price, 1988, Comparison of patiens with and without post-stroke major depression matched for size and location of lesion, Arch. Gen. Psychiatry 45, 247.

Starkstein, S.F., R.G. Robinson and T.R. Price, 1989, Affective disorders and cerebral vascular disease, Br. J. Psychiatry 154, 170.

Thomson, A.M., 1990, Glycine is a coagonist at the NMDA receptor/channel complex, Prog. Neurobiol. 35, 53.

Thiels, E., D.J. Weisz and T.W. Berger, 1992, In vivo modulation of *N*-methyl-D-aspartate receptor-dependent long-term potentiation by the glycine modulatory site, Neurosci. 46, 501.

Trullas, R. and P. Skolnick, 1990, Functional antagonists at the NMDA receptor complex exhibit antidepressant actions, Eur. J. Pharmacol. 185, 1.

Trullas, R., T. Folio, A. Young, R. Miller, K. Boje and P. Skolnick, 1991, 1-Aminocyclopropanecarboxylates exhibit antidepressant and anxiolytic actions in animal models, Eur. J. Pharmacol. 203, 379.

Van Harten, J., 1993, Clinical pharmacokinetics of selective serotonin reuptake inhibitors [Review], Clin. Pharmacokinet. 24, 203.

Vetulani, J., L. Antkiewicz-Michaluk, A. Rokosz-Pelc and A. Pilc, 1984, Alpha up-beta down adrenergic regulation: a possible mechanism of action of antidepressant treatments, Pol. J. Pharmacol. Pharm. 36, 231.

Williams, K., C. Romano, M. Dichter and P. Molinoff, 1991, Modulation of the NMDA receptor by polyamines, Life Sci. 48, 469.

Wood, P.L., M.R. Emmett, T.S. Rao, S. Mick, J. Cler and S. Iyengar, 1989, In vivo modulation of the *N*-methyl-D-aspartate receptor complex by D-serine: potentiation of ongoing neuronal activity as evidenced by increased cerebellar cyclic GMP, J. Neurochem. 53, 979.

Yoneda, Y., T. Suzuki, K. Ogita and D. Han, 1993, Support for radiolabeling of a glycine recognition domain on the *N*-methyl-D-aspartate receptor ionophore complex by 5,7-[^{3}H]dichlorokynurenate in rat brain, J. Neurochem. 60, 634.