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Short communication

Noradrenergic lesion antagonizes desipramine-induced adaptation of NMDA receptors

Andrew Harkin, Gabriel Nowak, Ian A. Paul *

Laboratory of Neurobehavioral Pharmacology and Immunology, Division of Neurobiology and Behavior Research, Department of Psychiatry, University of Mississippi Medical Center, Box 127, 2500 North State Street, Jackson, MS 39216-4505, USA

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Abstract

Repeated administration of the tricyclic antidepressant, desipramine, for 28 days to mice effected a decrease in the potency of glycine to displace [^{3}H]5,7-dichlorokynurenic acid (5,7-DCKA) in mouse cortical homogenates. Pre-treatment with the noradrenergic neurotoxin DSP-4, while having no effect alone, attenuated the desipramine-induced effect. The present findings support a norepinephrine-dependent adaptation of the NMDA receptor complex in vivo following chronic desipramine treatment. The inter-relationship of norepinephrine and glutamate transmission may provide insight into the mechanism underlying the action of antidepressant drugs.

Keywords: Antidepressant; NMDA receptor; Glycine; DSP-4; Norepinephrine; Desipramine

1. Introduction

It is widely reported that norepinephrine is involved in the pathophysiology of depression. Moreover, activity at noradrenergic neurons has been hypothesized to be responsible for the therapeutic actions of a number of antidepressants (reviewed by Redmond et al., 1997). While extensive research has found that chronic administration of a variety of antidepressant treatments result in down regulation of β-adrenoceptors, this adaptation does not appear to represent a common mechanism of antidepressant action (Heninger and Charney, 1987; Charney, 1998). More recently, we have demonstrated that chronic but not acute antidepressant treatment results in a reduction in both the potency of glycine to inhibit the binding of [^{3}H]5,7-dichlorokynurenic acid (5,7-DCKA) to strychnine insensitive glycine receptors (reviewed in Huang et al., 1997). Furthermore, antidepressant-induced increase in the IC_{50} of glycine to inhibit [^{3}H]5,7-DCKA binding to NMDA receptors, is a significantly better predictor of antidepressant drug activity than either β-adrenoceptor downregulation or efficacy in the forced swim test (Paul et al., 1994). Since antidepressants lead to an adaptive change to the binding properties of the NMDA receptor we hypothesized that noradrenergic function would be critical to the action of selective norepinephrine reuptake inhibitors such as desipramine at the NMDA receptor.

In the present study, mice were treated with *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) preceded by inhibition of the serotonin transporter to destroy noradrenergic neurons as previously described (Nowak et al., 1991; Hall et al., 1984). The ligand-binding properties of the strychnine-insensitive glycine recognition site of the NMDA receptor complex were assessed in vehicle and DSP-4 pre-treated animals following chronic desipramine administration. We now report that lesion of noradrenergic neurons blocked the increase in the IC_{50} of glycine to displace [^{3}H]5,7-DCKA binding to cortical membranes normally observed after chronic desipramine treatment. In contrast, lesion of noradrenergic neurons without desipramine treatment did not affect [^{3}H]5,7-DCKA binding or its displacement by glycine. These data demonstrate a functional link between antidepressant actions at noradrenergic neurons and the adaptation of the NMDA receptor.

* Corresponding author. Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505, USA. Tel.: +1-601-984-5998; fax: +1-601-984-5884.

E-mail address: ipaul@psychiatry.umsmed.edu (I.A. Paul).

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2. Methods

2.1. Subjects

Male NIH Swiss–Webster mice (Harlan Sprague–Dawley, Indianapolis, IN) weighing 25–30 g on arrival were used in this study. Mice were housed six per cage with ad libitum food and water. Lighting was controlled on a 12-h light:12-h dark cycle (lights on: 0600 h) and the facility temperature was maintained at 20 ± 2°C. Animals were given a week to acclimate to the animal facility prior to onset of the experiment.

2.2. Drug treatment

All animals received an injection of the selective serotonin reuptake inhibitor, citalopram (20 mg/kg, i.p., in 0.2 ml/mouse). Thirty minutes after receiving the injection of citalopram, mice received an injection of DSP-4 or vehicle in a volume of 6 ml/kg. The DSP-4 treatment was preceded by the administration of citalopram, in order to prevent serotonin depletion. The dose of DSP-4 (62.5 mg/kg) was selected from previous studies that used DSP-4 lesioning in mice (Finnegan et al., 1990; Yu et al., 1994). Two weeks following DSP-4 pre-treatment, desipramine (10 mg/kg) was dissolved in saline in an injection volume of 6 ml/kg and administered once daily for 4 weeks. All injections were given intraperitoneally (i.p.). Controls received injections of vehicle (saline) alone.

2.3. [^{3}H]5,7-DCKA displacement assays

Twenty-four hours after the last antidepressant treatment, all mice were sacrificed and the brains dissected, prepared, and assayed for [^{3}H]5,7-DCKA binding as previously described by Nowak et al. (1996). [^{3}H]5,7-DCKA is a high-affinity antagonist at strychnine-insensitive glycine receptors that is displaced by glycine concentrations comparable to those which allosterically modulate radioligand binding to other subunits of the NMDA receptor (Baron et al., 1991). Frontal and posterior cortices were dissected and placed on aluminium foil over solid CO_2. The frozen tissue was stored at -70°C for 1–2 weeks before assay. Tissues were initially thawed in 50 volumes of ice-cold 5 mM HEPES/4.5 mM Tris (HTS) buffer (pH 7.8) and homogenized using a Brinkmann Polytron (setting 6, 30 s) and centrifuged at $20{,}000 \times g$ for 20 min. Pellets were subsequently reconstituted in 50 volumes of fresh buffer and then centrifuged at $20{,}000 \times g$ for 20 min to eliminate endogenous substances (e.g., glycine, glutamate) that interfere with the radioligand-binding procedures. The tissue pellet obtained was washed once more in 50 volumes of HTS and twice in HTS containing 1mM EDTA. Following centrifugation ($20{,}000 \times g$ for 20 min), the pellet was reconstituted in 5–10 volumes of fresh HTS and stored at -70°C for at least 4 days before the assay. On the day of the assay, the frozen aliquot was thawed, re-suspended in 50 volumes of fresh HTS and centrifuged at $20{,}000 \times g$ for 20 min immediately before assay. The final tissue pellet was suspended in 50 mM HEPES–KOH buffer (pH 8). [^{3}H]5,7-DCKA binding was assayed in 50 mM HEPES–KOH buffer by incubating membranes (approx. 0.2 mg of protein) for 90 min at 0–4°C with 20 nM [^{3}H]5,7-DCKA (15.8 Ci/mmol) with buffer and/or drugs added to a final volume of 1 ml. Nonspecific binding was defined by using 100 μM of glycine. A total of 11 concentrations (10^{-4} to 10^{-9} M) of glycine were used to construct displacement curves. Incubations were terminated in a refrigerated centrifuge (20 min at $20{,}000 \times g$). After aspirating the supernatant, pellets were superficially washed once with 1 ml of 50 mM HEPES–KOH buffer, dissolved in tissue solubilizer and neutralized with glacial acetic acid. Scintillation cocktail (4 ml of Cytoscint) was added to this mixture and the radioactivity was measured in a Beckman (Fullerton, CA) LS 3801 liquid scintillation counter. Assays were performed in duplicate. Protein content was determined using the bicinchoninic acid method (Smith et al., 1985) with kits from Pierce (Rockford, IL).

2.4. [^{3}H]Nisoxetine binding

The extent of the lesion was determined by measurement of the specific binding of [^{3}H]nisoxetine, which is reported to bind selectively to presynaptic norepinephrine transporter/re-uptake site (Tejani-Butt, 1992). On the day of sacrifice, midbrain and cerebellum were dissected and placed on aluminium foil over solid CO_2. As the cortical tissue had been utilized in the [^{3}H]5,7-DCKA displacement assays, the midbrain and cerebellum were chosen as appropriate brain regions for [^{3}H]nisoxetine binding given the extensive innervation of norepinephrine neurons in these regions. The frozen tissue was stored at -70°C for 1–2 weeks before assay. On the day of assay, tissues were thawed in 50 volumes of ice-cold Tris pH 7.4, 120 mM NaCl, 5 mM KCl and homogenized using a Brinkmann polytron (setting 6, 15 s) and centrifuged at $20{,}000 \times g$ for 20 min. Pellets were reconstituted in 50 volumes of fresh buffer using a brief polytron pulse and centrifuged at $20{,}000 \times g$ for 20 min. The tissue pellet was washed once more in 50 volumes of ice-cold buffer, centrifuged at $20{,}000 \times g$ for 20 min and reconstituted in 50 volumes of ice-cold Tris pH 7.4, 300 mM NaCl, 5 mM KCl to approximately 1 mg of protein per ml.

[^{3}H]Nisoxetine binding was performed at a single concentration using a modified form of the method of Tejani-Butt (Tejani-Butt, 1992). Membrane suspensions (0.2–0.3 mg/assay) were incubated with 0.7 nM [^{3}H]nisoxetine (85.9 Ci/mmol) for 6 h at 0–4°C. Non-specific binding was determined with 100 μM desipramine. Assays were performed in triplicate and terminated by rapid filtration through Whatman GF/C filters presoaked in 0.03% polyethyleneimine. The filters were rinsed once with 5 ml

of ice-cold Tris buffer. The filters were placed in 0.5 ml of Microscint 20 scintillation cocktail. The radioactivity retained by the filters was measured in a Packard Topcount microplate scintillation counter.

2.5. Statistical analysis

Samples were assayed individually and the data for each subject were reduced initially by using iterative curve fitting routines to yield IC_{50} and specific binding (competitive inhibition Graph Pad version 2). A GB-STAT routine was used to analyze the data. Statistical comparisons were made initially by performing a two-way analysis of variance where DSP-4 and desipramine treatments were the first and second factors. Significant main or interactive effects were further analyzed using post hoc comparisons with a Fishers' least significant difference (LSD) multiple range test. Data were deemed significant when $P < 0.05$.

3. Results

Inhibition of 20 nM [^{3}H]5,7-DCKA binding to cortical membranes by glycine was consistent with a one-site competitive mechanism. The ANOVA of the IC_{50} of glycine to displace [^{3}H]5,7-DCKA revealed an effect of drug [$F(1,36) = 6.68$, $P = 0.013$], lesion [$F(1,36) = 6.42$, $P = 0.016$] and a drug × lesion interaction [$F(1,36) = 9.52$, $P = 0.004$]. Chronic desipramine treatment increased the IC_{50} of glycine to inhibit [^{3}H]5,7-DCKA binding in non-lesioned animals ($P < 0.01$, Fig. 1A, Table 1). The IC_{50} value of glycine increased 357% from 410 to 1872 nM following chronic desipramine treatment. This effect was completely antagonized in the DSP-4 + desipramine-treated group ($P < 0.01$, Fig. 1B and C, Table 1). The IC_{50} value for DSP-4 + vehicle-treated animals was unchanged when compared to vehicle + vehicle controls. There were no changes in the specific binding of [^{3}H]5,7-DCKA (Table 1).

The ANOVA of [^{3}H]nisoxetine binding to midbrain homogenates revealed an effect of DSP-4 pre-treatment [$F(1,36) = 6.12$, $P = 0.018$]. The specific binding of [^{3}H]nisoxetine in midbrain of DSP-4-treated subjects was reduced from 23.4 ± 6 to 13.6 ± 2.5 fmol/mg protein ($P < 0.05$) when compared to sham controls. The ANOVA of [^{3}H]nisoxetine binding to cerebellar homogenates shows an effect of DSP-4 pre-treatment [$F(1,36) = 23.63$, $P < 0.001$] and an effect of desipramine treatment [$F(1,36) = 8.15$, $P = 0.007$]. The specific binding of [^{3}H]nisoxetine in cerebellum from DSP-4-treated subjects was reduced from 15.2 ± 2.6 to 7.8 ± 0.7, and 5.0 ± 1.2 fmol/mg protein in sham vehicle ($P < 0.05$) and desipramine-treated ($P < 0.01$) groups, respectively. Desipramine treatment reduced [^{3}H]nisoxetine binding in the cerebellum from 15.2 ± 2.6

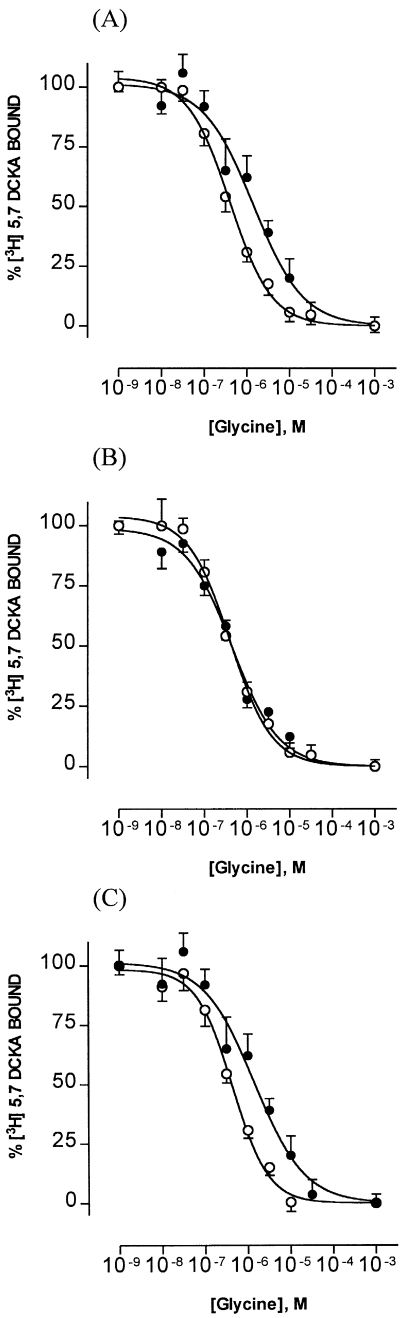

Fig. 1. Effect of chronic treatment with desipramine on glycine inhibition of [^{3}H] 5,7-DCKA binding with and without DSP-4 pre-treatment. (A) Effect of chronic treatment with desipramine (filled symbols) on glycine inhibition of [^{3}H]5,7-DCKA binding in cortex when compared to saline-treated controls (open symbols). Desipramine increased the IC_{50} of glycine to displace [^{3}H] 5,7-DCKA from cortical homogenates. (B) Effect of DSP-4 pre-treatment on glycine inhibition of [^{3}H]5,7-DCKA binding in cortex following chronic desipramine treatment (filled symbols) when compared to the vehicle + saline-treated group (open symbols). DSP-4 pre-treatment blocked the desipramine-induced increase in the IC_{50} of glycine to displace [^{3}H]5,7-DCKA. (C) There was no effect of DSP-4 pre-treatment (filled symbols) on glycine inhibition of [^{3}H]5,7-DCKA binding in cortex when compared to vehicle + saline-treated controls (open symbols). Data are expressed as the percentages specific binding and represent the means ± S.E.M. of 7–13 animals per group. Untransformed values are presented in Table 1.

Table 1
Effect of desipramine treatment in DSP-4-treated mice on glycine displacement of [^{3}H]5,7-DCKA in cortical homogenates
Animals were pre-treated with vehicle or DSP-4 (62.5 mg/kg, i.p.). Two weeks later, the animals were treated with saline or desipramine (10 mg/kg i.p.) once daily for 28 days.
Data is expressed as mean ± S.E.M. of 7–13 animals per group.

Pretreatment	Chronic treatment	Specific binding (fmol/mg protein)	IC_{50} (nM)	n_H
Vehicle	Saline	659 ± 56	410 ± 50	0.95 ± 0.12
DSP-4	Saline	623 ± 51	551 ± 128	0.99 ± 0.19
Vehicle	Desipramine	604 ± 81	1872 ± 569*	0.91 ± 0.19
DSP-4	Desipramine	630 ± 50	432 ± 99**	1.08 ± 0.15

*$P < 0.01$ vs. vehicle saline.
**$P < 0.01$ vs. vehicle desipramine (Fisher's LSD).

to 10.5 ± 1.1 fmol/mg when compared to saline-treated subjects ($P < 0.05$).

4. Discussion

In agreement with previous studies from this and other laboratories (Paul et al., 1994), the present study demonstrates that chronic desipramine treatment increases the IC_{50} of glycine to displace [^{3}H]5,7-DCKA in mouse cortical homogenates thereby reducing the potency of glycine on the NMDA receptor complex. However, chronic desipramine treatment was without effect on the potency of glycine to displace [^{3}H]5,7-DCKA in animals with noradrenergic lesions. These data demonstrate that intact noradrenergic function is required for desipramine to affect the NMDA receptor. In contrast, DSP-4 lesion alone did not affect the binding profile of [^{3}H]5,7 DCKA suggesting that tonic maintenance of the radioligand-binding characteristics of the NMDA receptor is maintained despite DSP-4-induced denervation.

This study demonstrated 50–60% reduction in [^{3}H]nisoxetine binding in mouse mid-brain and cerebellum following DSP-4 pre-treatment. Several previous studies have demonstrated that noradrenergic lesion with DSP-4 is relatively selective, destroying noradrenergic fibers originating in the locus coeruleus that project to cortex while comparatively sparing noradrenergic neurons originating in other areas (Logue et al., 1985; Grzanna et al., 1989; Fritschy and Grzanna, 1992). Our data are comparable to those of other laboratories employing DSP-4 reporting 70–80% depletions in frontal cortex and smaller depletions in midbrain and cerebellum (Heal et al., 1993; Theron et al., 1993). In addition, chronic desipramine treatment reduced [^{3}H]nisoxetine binding in the present study. In a previous autoradiographic study, Bauer and Tejani-Butt (1992) found that repeated desipramine treatment caused a significant decrease in the binding of [^{3}H]nisoxetine in 8 out of 17 brain regions (limbic) of rat brain.

DSP-4 inactivates nodarenergic neurons after being transported into them via a high affinity uptake system and subsequent destruction of presynaptic terminals (Hallman and Jonsson, 1984). DSP-4 thus destroys the site of action of desipramine and correspondingly blocks the desipramine-induced increase in the IC_{50} of glycine to displace [^{3}H]5,7-DCKA from cortical homogenates. By contrast DSP-4 lesioning alone does not affect the binding properties of [^{3}H]5,7-DCKA. While DSP-4 treatment typically results in 70–80% depletion of norepinephrine, preferentially in neocortex due to destruction of cell bodies in the locus coeruleus, receptor proliferation occurs in response to noradrenergic denervation (Heal et al., 1993). Previous studies have demonstrated this by quantifying α_2-adrenoceptors in the rat brain after short-term DSP-4 lesioning (Heal et al., 1993). Thus although the number of functional presynaptic adrenergic terminals are reduced after DSP-4 lesioning, the overall effect on the noradrenergic system may be masked by receptor proliferation in response to noradrenergic denervation. In the absence of a complete lesion, it is therefore possible that noradrenergic transmission would proceed following DSP-4 lesioning in response to denervation-induced receptor proliferation. These properties of DSP-4 lesioning would account for inhibition of the effects of desipramine as the site of action is destroyed, inhibiting the action of desipramine to elevate synaptic concentrations of norepinephrine.

Swim stress has been found to cause a lasting adaptation of the NDMA receptor complex, which is manifested as increased potency of glycine to displace [^{3}H]5,7-DCKA from the strychnine insensitive glycine site of the NMDA receptor complex (Nowak et al., 1995). More recent studies on adaptive changes to the NMDA receptor complex have shown that exposure of rats to a chronic unpredictable mild stress regimen increases the potency of glycine to displace [^{3}H]5,7-DCKA in an imipramine-reversible manner (Paul, 1997). Such findings are consistent with an hypothesis that animal models of depression will produce adaptation of the NMDA receptor complex that is reversible by chronic treatment with antidepressant drugs. Although release and transient depletion of norepinephrine is a consequence of exposure to stress, the similarities between this and a noradrenergic lesion are not clear. Studies are ongoing to test the noradrenergic dependency of stressor-induced adaptation to the NDMA receptor complex. However in the present study, DSP-4 did not cause any change to the binding properties of 5,7-DCKA, indi-

cating that it did not produce any stress-like effect in this parameter. In addition, previous studies indicate that lesion with DSP-4 does not alter HPA axis functioning, indicating that DSP-4 does not produce a stress-like activation of the HPA axis (Dooley et al., 1984; Bakke et al., 1986). Therefore from our observations and those of others, it is our view that DSP-4 lesioning does not produce a stress-like state in laboratory animals.

The mechanism by which desipramine alters the potency of glycine at the NMDA receptor is presently unknown. The neurobiological mechanisms by which antidepressants in general achieve alterations in the radioligand-binding characteristics of the NMDA receptor are unclear. While some antidepressants directly interact with the complex (Reynolds and Miller, 1988; Kitamura et al., 1991), their affinity for the receptor is considered too low to account for the effects reported. Moreover, this explanation cannot be reconciled with the observation that electroconvulsive shock, a non-pharmacological therapy, is the most efficacious treatment to induce the alterations (Paul et al., 1993, 1994). Direct receptor interaction is unlikely to account for the effects that we observe. The in vitro effects of antidepressants currently suspected to be involved in their clinical efficacy include actions at monoaminergic neurons and their projections. Thus, antidepressant-induced adaptations to the NMDA receptor complex may be secondary to antidepressant action at monoaminergic terminals. Multiple lines of evidence have demonstrated that norepinephrine can modulate glutamatergic neurotransmission (Mori-Okamoto et al., 1991). Norepinephrine reduces glutamate release from rat cortical and spinal cord synaptosomes and reduces glutamatergic transmission in the entorhinal cortex via presynaptic α_2-adrenoceptors (Kamisaki et al., 1991). Conversely, activation of β-adrenoceptors, or direct activation of adenylate cyclase by forskolin, increases glutamate release in the hippocampus (Chavez-Noriega and Stevens, 1994; Gereau and Conn, 1994). Such studies indicate that noradrenergic neurons modulate glutamatergic transmission. Alterations to the strychnine insensitive glycine site on the NMDA receptor complex induced by chronic administration of desipramine, may thus be accounted for through action at noradrenergic nerve terminals. Our current hypothesis is that antidepressant interactions with monoaminergic neurotransmitter systems are required in order for these treatments to effect adaptation of the NMDA receptor complex.

Further studies using additional doses, other noradrenergic reuptake inhibitors in conjunction with using selective lesions to the serotonergic and dopaminergic systems will be required to determine whether the action of other antidepressant drugs at monoamine terminals is necessary for the subsequent adaptive binding changes to the NMDA receptor complex. Nonetheless, the results of the present study provide the first evidence for noradrenergic involvement in the adaptation of NMDA receptors following chronic desipramine treatment. As such they provide initial evidence to connect the recent discoveries relating antidepressant-induced NMDA receptor adaptation to a monoaminergic-dependent mechanism.

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