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Nitric oxide synthase inhibitors have antidepressant-like properties in mice
1. Acute treatments are active in the forced swim test

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Abstract

Previous studies have demonstrated that antagonists at the NMDA receptor are as efficacious as tricyclic antidepressants in pre-clinical antidepressant screening procedures and in blocking or reversing the behavioral deficits associated with animal analogs of major depressive symptomatology. The NMDA receptor complex gates Ca^{2+}, which interacts with calmodulin to subsequently activate nitric oxide (NO) synthase. We hypothesized that NO synthase antagonists might display antidepressant-like properties, similar to NMDA receptor antagonists. We examined the effects of N^G-nitro-L-arginine (L-NNA), its dextrorotatory enantiomer, D-NNA, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) at doses from 1 to 30 mg/kg in the forced swim test in mice. We now report that NO synthase antagonists are as efficacious as imipramine (15 mg/kg) in reducing the duration of immobility in the mouse forced swim test. The effects of NO synthase antagonists, as well as those of imipramine were blocked by pre-treatment with L-arginine (L-Arg) (500 mg/kg). In contrast to imipramine, the NO synthase antagonists were without effect on locomotor activity over the dose range active in the forced swim test (3–10 mg/kg). Likewise, L-Arg was without effect on locomotor activity. These data support the hypothesis that NO synthase antagonists possess antidepressant properties and may represent a novel class of therapeutics for major depressive disorders.

Keywords: Nitric oxide (NO) synthase; Antidepressant; Forced swimming; L-Arginine; N^G-Nitro-L-arginine; N^G-Monomethyl-L-arginine; N^G-Nitro-L-arginine methyl ester

1. Introduction

Recent in vivo and ex vivo findings indicate that the NMDA receptor complex may be a locus of antidepressant action. Functional antagonists of the NMDA receptor complex including a competitive NMDA receptor antagonist (2-amino-7-phosphonoheptanoic acid; AP-7) (Watkins and Olverman, 1987), a glycine partial agonist (1-aminocyclopropanecarboxylic acid; ACPC) (Marvizon et al., 1989; Watson and Lanthorn, 1990), and a use dependent channel antagonist (dizocilpine) (Wong et al., 1986) are as efficacious as tricyclic antidepressants in pre-clinical tests predictive of antidepressant activity (Trullas and Skolnick, 1990; Trullas et al., 1991; Maj et al., 1992b; Skolnick et al., 1992). Similarly, dizocilpine and the NMDA receptor antagonist DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentonoic acid (CGP-37849) block the behavioral effects of two putative animal models of depression, learned helplessness and chronic mild stress-induced deficits in sucrose consumption (Meloni et al., 1993; Papp and Moryl, 1993a,b). Moreover, a chronic regimen of either ACPC or dizocilpine produces a reduction in the density ('downregulation') of cortical β-adrenoceptors in mice comparable to that produced by the prototypic tricyclic antidepressant, imipramine (Paul et al., 1992). Likewise, Klimek and Papp (1994) have reported that chronic dizocilpine treatment downregulates cortical β-adrenoceptors, as well as 5-HT_2 receptors in rats. Thus, in both behavioral and biochemical screening procedures, antagonists at the NMDA receptor complex behave in a manner comparable to clinically active antidepressants.

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The NMDA receptor is a ligand-gated, voltage-sensitive ionophore which gates Ca^{2+} and, to a lesser extent, Na^{+} and K^{+} (Meguro et al., 1992). Stimulation of the receptor and opening of the ionophore results in Ca^{2+} entry into the receptive neuron. The Ca^{2+} binds to and stimulates a calcium–calmodulin complex which, in turn, stimulates nitric oxide (NO) synthesis (Southam and Garthwaite, 1993) to convert L-arginine (L-Arg) to L-citrulline and liberate NO. The released NO stimulates a NO-sensitive guanylyl cyclase to convert GTP to cGMP. NO synthase, the enzyme responsible for the production of NO can be exogenously stimulated with compounds such as sodium nitroprusside, *S*-nitroso-*N*-acetylpenicillamine and peripherally administered L-Arg. Conversely, NO synthase activity can be inhibited with N^{G}-nitro-L-arginine (L-NNA), N^{G}-nitro-L-arginine methyl ester (L-NAME) and N^{G}-monomethyl-L-arginine (L-NMMA).

Since NMDA receptor antagonists possess significant antidepressant-like properties (reviewed in (Trullas, 1997)), we hypothesized that NO synthase inhibitors would possess antidepressant properties. We have examined the effects of NO synthase antagonists in a pre-clinical behavioral screening procedure sensitive to antidepressant activity (Porsolt et al., 1977). We now report that NO synthase inhibitors dose-dependently and stereoselectively reduce the duration of immobility in mice in the forced swim test to a level comparable to that of the antidepressant, imipramine. The effects of NO synthase antagonists in the forced swim test are observed at doses that are devoid of locomotor stimulatory properties. Moreover, stimulation of NO synthase not only antagonized the behavioral effect of NO synthase inhibitors but also of the prototypic antidepressant, imipramine.

2. Methods

2.1. Subjects

Male NIH Swiss-Webster mice, 20–25 g on arrival were housed in groups of five under standard colony conditions with a 12 h light:dark cycle (lights on at 0700 h) and ad libitum food and water. All animals were allowed to acclimate to the colony for at least 7 days prior to any experimentation.

On the day of testing, mice were removed from the colony and allowed to acclimate to a sound-attenuated behavioral testing room for 2–3 h prior to testing. All testing was conducted between 1200 and 1700 h.

2.2. Forced swim test

The test was conducted using the method of Porsolt et al. (1977). Briefly, mice were individually placed in 6–7 cm of ambient temperature water (~22°C) in 2000 ml glass beakers. Mice were allowed to swim for 6 min and their activity was videotaped. Subsequently, the videotaped behavior was analyzed by one to two raters blind to treatment. The duration of immobility defined as the absence of active, escape-oriented behaviors such as swimming, jumping, rearing, sniffing or diving was recorded during the last 240 s of the test using the PORSOLT data collection program (Infallible Software, Res. Tri. Pk., NC). Each rater timed two mice per trial. Interrater variability was negligible, as was intertrial variability.

2.3. Open field locomotor activity

Mice were placed individually in the center of a circular open field (1 m diameter) and allowed to freely explore the field for 6 min. Activity was recorded on videotape and analyzed using the Videomex-V tracking system (Columbus Instruments, Columbus, OH).

2.4. Drug administration

All drugs were dissolved in saline (0.9% NaCl) and administered i.p. in a volume of 0.1 ml/mouse. Drugs were administered 60 min prior to testing with the exception of L-Arg which, was administered 10 min prior to testing. All drugs were obtained from Research Biochemicals (Natick, MA) and were prepared fresh on the day of testing.

2.5. Data analysis

Data were initially analyzed using a one- or two-factor analysis of variance wherein drug treatment and pre-treatment were the first and second factors, respectively. Individual group differences were assessed with Fisher's Least

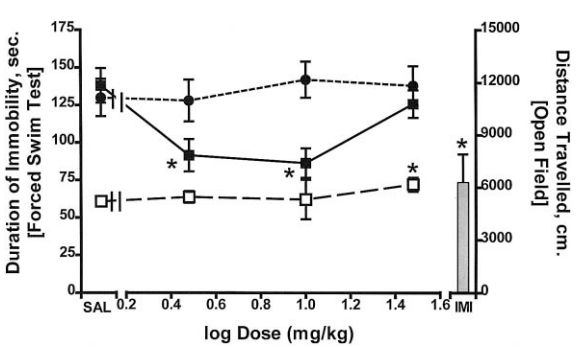

Fig. 1. N^{G}-nitroarginine effects on the duration of immobility in the forced swim test (filled symbols) and on activity in the open field (open symbols). Squares: N^{G}-nitro-L-arginine. Circles: N^{G}-nitro-D-arginine. Filled bar: imipramine (15 mg/kg). Data represent the mean ± S.E.M. of 9–10 animals per group. $^{*}P < 0.05$ vs. saline, Fisher's LSD.

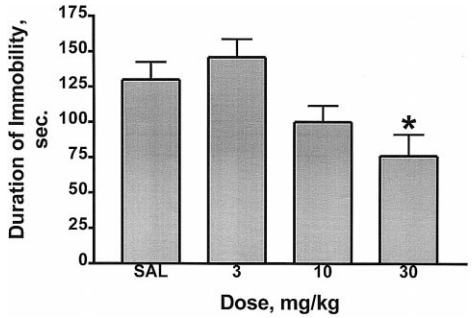

Fig. 2. Effect of N^G-monomethyl-L-arginine on the duration of immobility in the forced swim test. Data represent the mean ± S.E.M. of 9–10 mice per group. $^*P < 0.01$ vs. saline, Fisher's LSD.

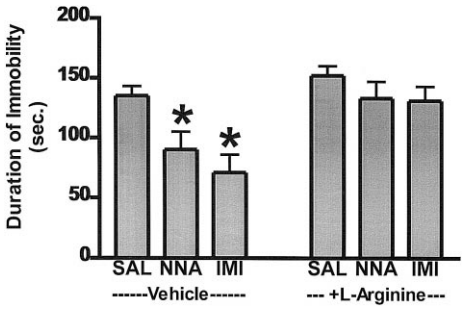

Fig. 3. L-Arg (LA—500 mg/kg) reversal of the effects of N^G-nitro-L-arginine (NNA—10 mg/kg) and imipramine (15 mg/kg) in the forced swim test. Data represent the mean ± S.E.M. of 9–10 subjects per group. $^*P < 0.01$ vs. saline, Fisher's LSD.

Significant Difference (LSD) test. Data were deemed significant when $P < 0.01$.

3. Results

L-NNA resulted in a dose-dependent, U-shaped reduction in the duration of immobility in the forced swim test with a maximum reduction of 38% at the 10 mg/kg dose compared to controls (Fig. 1). At 30 mg/kg, the duration of immobility of L-NNA-treated mice did not differ from controls. The efficacy of L-NNA (10 mg/kg) was comparable to that of a maximally effective dose of imipramine (−47% at 15 mg/kg). In contrast, L-NNA was a weak stimulator of locomotor activity and only achieved significant stimulation at the 30 mg/kg dose (Figs. 1 and 4).

Like L-NNA, both L-NMMA and L-NAME were dose-dependently efficacious in the forced swim test (Fig. 2, Table 1). While L-NMMA was equipotent with L-NNA, L-NAME was ~tenfold less potent than either L-NNA or L-NMMA. In contrast, the dextrorotatory isomer of L-NNA, D-NNA, was without effect in the forced swim test at doses up to 100 mg/kg (Fig. 1, Table 1). Moreover, at these doses, L-NMMA and L-NAME did not alter locomotor activity as assessed by total arm crossings in an elevated plus maze (data not shown).

Pre-treatment of mice with the NO synthase substrate, L-Arg alone was without significant effect on the duration of immobility in the forced swim test (Fig. 3, Table 2). However, L-Arg pre-treatment antagonized the ability of L-NNA (10 mg/kg) to reduce immobility in the forced

Table 1
Effect of NO synthase antagonists in the mouse forced swim test

Drug	Dose, mg/kg	Duration of immobility, s	Percentage control immobility
Saline	–	138 ± 12	100
Imipramine	15	74 ± 19	53*
L-NNA	1	122 ± 19	88
	3	92 ± 11	66*
	10	86 ± 10	63*
	30	126 ± 9	91
Saline	–	130 ± 13	100
D-NNA	3	128 ± 14	99
	10	142 ± 12	109
	30	138 ± 13	106
L-NMMA	3	146 ± 13	112
	10	100 ± 12	77
	30	76 ± 15	59*
L-NAME	30	128 ± 13	99
	100	93 ± 11	72*
	175	132 ± 14	102
	300	179 ± 10	138*

Data represent the mean ± S.E.M. of 10 mice/group.
Imipramine and L-NNA were run in a single experiment and D-NNA, L-NMMA and L-NAME in a second experiment.
Separate saline controls are presented for each experiment.
In the first experiment, single factor analysis of variance revealed a significant main effect of group [$F(5,50) = 4.05$, $P = 0.004$]. Likewise, in the second experiment, single factor analysis of variance revealed a significant main effect of group [$F(12,128) = 3.93$, $P < 0.001$].
Data for L-NAME at 3 and 10 mg/kg did not differ from control and are not shown.
Untransformed data are the same as those displayed in Figs. 1 and 2 and are included for clarity.
$^*P < 0.01$ Fisher's LSD following single factor ANOVA.

Table 2
Effect of L-Arg treatment in the mouse forced swim test

Pre-treatment	Acute treatment	Duration of immobility, s	Percentage SAL/SAL control
Saline	Saline	131 ± 8	100
L-Arg	Saline	152 ± 8	116
Saline	L-NNA	97 ± 15	74[a]
L-Arg	L-NNA	133 ± 14	102[b]
Saline	Imipramine	68 ± 15	52[a]
L-Arg	Imipramine	131 ± 12	100[b]

Data represent the mean ± S.E.M. of 10 mice/group.
Untransformed data are the same as those displayed in Fig. 3 and are included for clarity.
Two factor (Pre-treatment × acute treatment) analysis of variance revealed a main effect of pre-treatment [$F(2,54) = 6.05$, $P = 0.004$] and of acute treatment [$F(1,54) = 16.0$, $P < 0.001$].
[a]$P < 0.01$ vs. acute saline, Fisher's LSD following two factor ANOVA.
[b]$P < 0.01$ vs. saline pre-treatment.

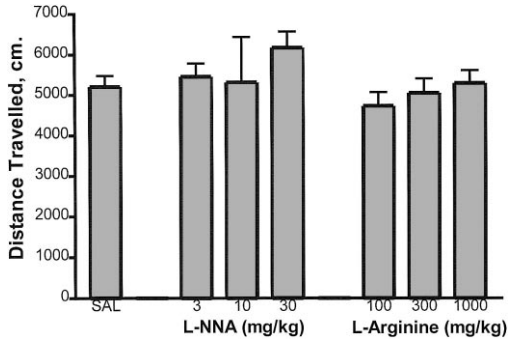

Fig. 4. Effect of N^G-nitro-L-arginine (L-NNA) and L-Arg on activity in the open field. Data represent the mean ± S.E.M. of 9–10 animals per group. ANOVA revealed no significant effects of treatment.

swim test (Fig. 3, Table 2). Moreover, L-Arg pre-treatment antagonized the effect of imipramine (15 mg/kg) in this test (Fig. 3, Table 2). In contrast, L-Arg was without effect on locomotor activity at any dose tested (Fig. 4).

4. Discussion

The present studies demonstrate that NO synthase antagonists possess antidepressant-like behavioral properties at doses that are without effect on locomotor activity. These effects are dose-dependent and stereoselective and can be reversed by co-treatment with the NO synthase agonist, L-Arg. Moreover, these studies have demonstrated that the effects of a clinically effective antidepressant, imipramine, can also be blocked by pre-treatment with L-Arg.

Support for a pharmacological interpretation of the effects of NO synthase antagonists in the forced swim test is found by relating the potency of the behavioral effects of the NO synthase inhibitors to their in vitro potencies. In vitro, L-NNA has low micromolar affinity for purified brain NO synthase, which may be competitively antagonized by L-Arg. Although an apparently less potent inhibitor in vivo, L-NMMA exhibits a similar affinity for brain NO synthase as the nitro derivative. However, the methyl derivative also appears to be a substrate of NO synthase, which is converted into stoichiometric amounts of NO and L-citrulline (Klatt et al., 1994). Klatt et al. suggest that L-NMMA behaves like a partial agonist of NO synthase. L-NAME is more potent than L-NMMA as an inhibitor of rat cerebellar NO synthase (Lambert et al., 1991). By contrast, Knowles et al. (1989) observed that L-NAME and L-NMMA were approximately equal in potency using a partially purified cytoplasmic preparation of NO synthase from the rat cortex. Other studies on the measurement of inhibition of brain NO synthase activity indicate that L-NNA is more potent than L-NAME after systemic administration (Carreau et al., 1994; Traystman et al., 1995). In addition, of the three L-Arg derived inhibitors of NO synthase, L-NNA has been reported as being the most selective in inhibiting brain NO synthase (Lambert et al., 1991). Thus, the potencies of L-Arg derived NO synthase inhibitors parallel the relative behavioral activity of these compounds, consistent with the involvement of NO synthase in the antidepressant-like effects in the forced swim test.

Reversal of the effects of L-NNA by L-Arg further support this, as L-Arg competes with L-NNA for active sites on NO synthase and prevents inhibition of the conversion of L-Arg to NO and L-citrulline (Rees et al., 1990). Several studies are consistent with a stereospecific reversal of the effects of NO synthase inhibitors by arginine and associated inhibition of peripheral and central functions (Palmer et al., 1988; Bluthe et al., 1992; Tseng et al., 1992; McDonald et al., 1994). In general, antagonism is associated with high doses of L-Arg requiring several fold excess of the substrate to obtain a complete reversal. In the present study, as arginine has poor access to the brain following peripheral administration, the selection of a single high dose of L-Arg was deemed appropriate. The effective dose ratio between L-NNA and L-Arg is compatible with relative NO synthase inhibition and reversal following systemic administration of the compounds.

NO regulates NMDA receptor activity in a biphasic manner playing both a positive (via activation of guanylate cyclase) and negative (via feedback effects on the NMDA receptor resulting in decreased NMDA receptor and NO synthase activity) modulatory role in NMDA receptor mediated events (Lei et al., 1992; Manzoni and Bockaert, 1993). Such opposing effects of NO might explain why NO synthase inhibitors exert an inverted U-shaped or biphasic dose response curve in the forced swim test relating to the facilitatory and inhibitory effect of NO on the NMDA receptor. In addition, such mechanisms may help explain why so many contradictory results have been found suggesting that NO either facilitates or inhibits NMDA receptor mediated events such as long-term potentiation (Izumi et al., 1992), seizures (Rundfeldt et al., 1995), nociception (Kawabata et al., 1993), neurotoxicity (Weissman et al., 1992) and anxiolysis (De Oliveira et al., 1997).

Alternatively, the biphasic effect of some NO synthase antagonists, such as L-NAME (300 mg/kg), may be attributed to adverse effects of high doses of the compound such as impaired cerebrovascular circulation (L-NAME is reported as being a prototypical endothelial NO synthase inhibitor) or loss of control over locomotion and coordination (L-NAME is estimated at being 200 times more potent than L-NMMA as an inhibitor of adult rat cerebellar NO synthase (see Lambert et al., 1991)). Similarly, recent observations with high doses of 7-nitroindazole administration to mice (≥ 300 mg/kg, i.p.) producing loss of locomotion, coordination and righting reflex, are possibly attributable to inhibition of neuronal NO synthase in the cerebellum.

Reuptake of biogenic amines are of primary importance in terminating the action of these transmitters on their receptor sites. It follows from this that a drug that inhibits the reuptake mechanism could prolong the physiological and behavioral changes associated with the neurotransmitter. The prototypic tricyclic antidepressant, imipramine, is a potent inhibitor of neuronal norepinephrine and serotonin reuptake (Glowinski and Axelrod, 1964; Corrodi and Fuxe, 1968). However, whether the activity of imipramine in the forced swim test is associated with increased noradrenergic and serotonergic activity is unknown. Some studies have attempted to show that various antidepressants including tricyclic antidepressants can reduce the duration of immobility in the swim test by activating catecholaminergic mechanisms in the brain (Zebrowska-Lupina, 1980; Borsini et al., 1981; Plaznik et al., 1985); however, the support for such a mechanism is equivocal. More recent evidence that norepinephrine is not involved is argued by the observation that there are no changes to the anti-immobility activity of the desipramine following 6-hydroxydopamine and (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine) DSP-4 induced destruction of brain noradrenergic neurons. (Esposito et al., 1987; Danysz et al., 1989).

A role for the glutamatergic system in the antidepressant effect of tricyclic antidepressants and other antidepressants appears particularly relevant given the qualitative similarities between NMDA receptor antagonists, NO synthase inhibitors (as shown in the present study) and tricyclic antidepressants in animal models and other biochemical tests predictive of antidepressant activity (Trullas and Skolnick, 1990; Paul et al., 1992; Meloni et al., 1993; Papp and Moryl, 1993b; Redmond et al., 1997). Moreover, combined treatment with the non-competitive NMDA receptor antagonist, dizocilpine and imipramine at doses that are ineffective alone results in an antidepressant-like response in the forced swim test (Maj et al., 1992a). Furthermore, the 5-HT reuptake inhibitor, citalopram, when given alone is inactive yet when combined with a subactive dose of dizocilpine is able to produce an antidepressant-like response in the test. Maj et al. suggest that a synergism exists between antidepressants and dizocilpine and that interactions between aminergic and glutamatergic transmission are likely to underlie the enhanced responses. The present study supports a role for NO synthase inhibition in the antidepressant effects of imipramine. Whether tricyclic antidepressants affect glutamatergic/NO transmission directly or indirectly by blockade of the reuptake of amines is unclear and is the subject of ongoing investigation. Nevertheless, the present experiments not only support the antidepressant activity of NO synthase inhibitors but also suggest that NMDA/NO systems may have a role in the antidepressant effects of imipramine in the forced swim paradigm.

As noted above, activation of NO synthase is part of the cascade of subcellular events leading from activation of the NMDA receptor to stimulation of guanylyl cyclase and post-synaptic activation. We have hypothesized that interruption of this cascade at any point will result in antidepressant-like activity. The present results with NO synthase antagonists are consistent with this hypothesis as are the previous observations that NMDA receptor antagonists possess antidepressant-like activity (see Trullas, 1997 for a review).

In conjunction with our evidence that NO synthase inhibitors have antidepressant activity, a potential role for NO in affective disorders has also recently been proposed (Harvey, 1996). NO is a regulator of both short- and long-term neuronal adaptive changes and consequently, may play a role in neuronal adaptation to antidepressant drugs. Targets of NO include guanylate cyclase, G proteins, amino acid, amine and neuropeptide release and transport. NO mediated cGMP synthesis also mediates induction of immediate early gene expression which is implicated in long-term synaptic changes and more recently in the mechanism of action of antidepressant drugs (reviewed by Harvey, 1996; see also Duman et al., 1997). Inhibitors of NO synthase are beginning to be developed for the various isoforms of NO synthase, which raises the possibility of developing well-tolerated and selective NO synthase inhibitors that may be used for the treatment of CNS disorders where NO is implicated.

The present studies diverge from previous studies of the actions of NMDA receptor antagonists on one significant point. We have demonstrated in the present studies that L-Arg treatment can antagonize the immobility-reducing effects of *both* NO synthase antagonists *and* imipramine in the forced swim test. In previous studies of the effects of an antagonist at the strychnine-insensitive recognition site of the NMDA receptor (1-aminocyclopropanecarboxylic acid, ACPC), Trullas and co-workers demonstrated that the effects of ACPC in the forced swim test could be blocked by pre-treatment with glycine. In contrast, glycine pre-treatment was without effect on the reduction in immobility produced by imipramine treatment (Trullas and Skolnick, 1990; Trullas et al., 1991). Thus, while the effects of imipramine in the forced swim test are not dependent on antagonism of the NMDA receptor, they apparently are dependent on antagonism of NO synthase.

The present studies demonstrate that antagonists of NO synthase possess significant antidepressant-like features that are dose-dependent, stereoselective and reversible by L-Arg. Together, these data demonstrate that antagonists of NO synthase represent a novel class of potential therapeutic agents for the treatment of major depression and suggest that interruption of the production NO by NO synthase may be critical to the action of antidepressants.

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References

Bluthe, R.M., Sparber, S., Dantzer, R., 1992. Modulation of the behavioural effects of interleukin-1 in mice by nitric oxide. NeuroReport 3, 207–209.

Borsini, F., Bendotti, C., Velkov, V., Rech, R., Samanin, R., 1981. Immobility test: effects of 5-hydroxytryptaminergic drugs and role of catecholamines in the activity of some antidepressants. J. Pharm. Pharmacol. 33, 33–37.

Carreau, A., Duval, D., Poignet, H., Scatton, B., Vige, X., Nowicki, J.P., 1994. Neuroprotective efficacy of *N*-omega-nitro-L-arginine after focal cerebral ischemia in the mouse and inhibition of cortical nitric oxide synthase. Eur. J. Pharmacol. 256, 241–249.

Corrodi, H., Fuxe, K., 1968. The effect of imipramine on central monoamine neurons. J. Pharm. Pharmacol. 20, 230–231.

Danysz, W., Kostowski, W., Hauptmann, M., Bidzinski, A., 1989. Are ascending noradrenergic and serotonergic pathways necessary for effects of electroconvulsive treatment? Clonidine hypothermia and forced swim study. Pol. J. Pharmacol. Pharm. 41, 15–22.

De Oliveira, C.L., Del Bel, E.A., Guimaraes, F.S., 1997. Effects of L-NOARG on plus-maze performance in rats. Pharmacol. Biochem. Behav. 56, 55–59.

Duman, R.S., Heninger, G.R., Nestler, E.J., 1997. A molecular and cellular theory of depression. Arch. Gen. Psychiatry 54, 597–606.

Esposito, E., Ossowska, G., Samanin, R., 1987. Further evidence that noradrenaline is not involved in the anti-immobility activity of chronic desipramine in the rat. Eur. J. Pharmacol. 136, 429–432.

Glowinski, J., Axelrod, J., 1964. Inhibition of tritiated noradrenaline in intact rat brain by imipramine and structurally related compounds. Nature 204, 1318–1319.

Harvey, B.E., 1996. Affective disorders and nitric oxide: a role in pathways to relapse and refractoriness?. Human Psychopharmacology 11, 309–319.

Izumi, Y., Clifford, D.B., Zorumski, C.F., 1992. Inhibition of long-term potentiation by NMDA-mediated nitric oxide release. Science 257, 1273–1276.

Kawabata, A., Umeda, N., Takagi, H., 1993. L-arginine exerts a dual role in nociceptive processing in the brain: involvement of the kyotorphin–Met–enkephalin pathway and NO–cyclic GMP pathway. Br. J. Pharmacol. 109, 73–79.

Klatt, P., Schmidt, K., Brunner, F., Mayer, B., 1994. Inhibitors of brain nitric oxide synthase. Binding kinetics, metabolism, and enzyme inactivation. J. Biol. Chem. 269, 1674–1680.

Klimek, V., Papp, M., 1994. The effect of MK-801 and imipramine on beta-adrenergic and 5-HT2 receptors in the chronic mild stress model of depression in rats. Pol. J. Pharmacol. 46, 67–69.

Knowles, R.G., Palacios, M., Palmer, R.M., Moncada, S., 1989. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase. Proc. Natl. Acad. Sci. U.S.A. 86, 5159–5162.

Lambert, L.E., Whitten, J.P., Baron, B.M., Cheng, H.C., Doherty, N.S., McDonald, I.A., 1991. Nitric oxide synthesis in the CNS endothelium and macrophages differs in its sensitivity to inhibition by arginine analogues. Life Sci. 48, 69–75.

Lei, S.Z., Pan, Z.H., Aggarwal, S.K., Chen, H.S., Hartman, J., Sucher, N.J., Lipton, S.A., 1992. Effect of nitric oxide production on the redox modulatory site of the NMDA receptor–channel complex. Neuron 8, 1087–1099.

Maj, J., Rogoz, Z., Skuza, G., 1992a. The effects of combined treatment with MK-801 and antidepressant drugs in the forced swimming test in rats. Pol. J. Pharmacol. Pharm. 44, 217–226.

Maj, J., Rogoz, Z., Skuza, G., Sowinska, H., 1992b. Effects of MK-801 and antidepressant drugs in the forced swimming test in rats. Eur. J. Neuropsychopharmacol. 2, 37–41.

Manzoni, O., Bockaert, J., 1993. Nitric oxide synthase activity endogenously modulates NMDA receptors. J. Neurochem. 61, 368–370.

Marvizon, J.C., Lewin, A.H., Skolnick, P., 1989. 1-Aminocyclopropane carboxylic acid: a potent and selective ligand for the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex. J. Neurochem. 52, 992–994.

McDonald, C.E., Gagnon, M.J., Ellenberger, E.A., Hodges, B.L., Ream, J.K., Tousman, S.A., Quock, R.M., 1994. Inhibitors of nitric oxide synthesis antagonize nitrous oxide antinociception in mice and rats. J. Pharmacol. Exp. Ther. 269, 601–608.

Meguro, H., Mori, H., Araki, K., Kushiya, E., Kutsuwada, T., Yamazaki, M., Kumanishi, T., Arakawa, M., Sakimura, K., Mishina, M., 1992. Functional characterization of a heteromeric NMDA receptor channel from cloned cDNAs. Nature 357, 70–74.

Meloni, D., Gambarana, C., De Montis, M.G., Dal Pra, P., Taddei, I., Tagliamonte, A., 1993. Dizocilpine antagonizes the effect of chronic imipramine on learned helplessness in rats. Pharmacol. Biochem. Behav. 46, 423–426.

Palmer, R.M., Rees, D.D., Ashton, D.S., Moncada, S., 1988. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem. Biophys. Res. Commun. 153, 1251–1256.

Papp, M., Moryl, E., 1993a. New evidence for the antidepressant activity of MK-801, a non-competitive antagonist of NMDA receptors. Pol. J. Pharmacol. 45, 549–553.

Papp, M., Moryl, E., 1993b. 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, 348–349.

Paul, I.A., Trullas, R., Skolnick, P., Nowak, G., 1992. Down-regulation of cortical beta-adrenoceptors by chronic treatment with functional NMDA antagonists. Psychopharmacology (Berl) 106, 285–287.

Plaznik, A., Danysz, W., Kostowski, W., 1985. Mesolimbic noradrenaline but not dopamine is responsible for organization of rat behavior in the forced swim test and an anti-immobilizing effect of desipramine. Pol. J. Pharmacol. Pharm. 37, 347–357.

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327–336.

Redmond, A.M., Kelly, J.P., Leonard, B.E., 1997. Behavioural and neurochemical effects of dizocilpine in the olfactory bulbectomized rat model of depression. Pharmacol. Biochem. Behav. 58, 355–359.

Rees, D.D., Palmer, R.M., Schulz, R., Hodson, H.F., Moncada, S., 1990. Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101, 746–752.

Rundfeldt, C., Koch, R., Richter, A., Mevissen, M., Gerecke, U., Loscher, W., 1995. Dose-dependent anticonvulsant and proconvulsant effects of nitric oxide synthase inhibitors on seizure threshold in a cortical stimulation model in rats. Eur. J. Pharmacol. 274, 73–81.

Skolnick, P., Miller, R., Young, A., Boje, K., Trullas, R., 1992. Chronic treatment with 1-aminocyclopropanecarboxylic acid desensitizes behavioral responses to compounds acting at the *N*-methyl-D-aspartate receptor complex. Psychopharmacology (Berl) 107, 489–496.

Southam, E., Garthwaite, J., 1993. The nitric oxide–cyclic GMP signalling pathway in rat brain. Neuropharmacology 32, 1267–1277.

Traystman, R.J., Moore, L.E., Helfaer, M.A., Davis, S., Banasiak, K., Williams, M., Hurn, P.D., 1995. Nitro-L-arginine analogues. Dose- and time-related nitric oxide synthase inhibition in brain. Stroke 26, 864–869.

Trullas, R., 1997. Functional NMDA antagonists: a new class of antidepressant agents. In: Skolnick, P. (Ed.), Antidepressants: New Pharmacological Strategies. Humana Press, Totowa, NJ, pp. 103–124.

Trullas, R., Skolnick, P., 1990. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. Eur. J. Pharmacol. 185, 1–10.

Trullas, R., Folio, T., Young, A., Miller, R., Boje, K., Skolnick, P., 1991. 1-aminocyclopropanecarboxylates exhibit antidepressant and anxiolytic actions in animal models. Eur. J. Pharmacol. 203, 379–385.

Tseng, L.F., Xu, J.Y., Pieper, G.M., 1992. Increase of nitric oxide production by L-arginine potentiates i.c.v. administered beta-endorphin-induced antinociception in the mouse. Eur. J. Pharmacol. 212, 301–303.

Watkins, J.C., Olverman, H.J., 1987. Agonists and antagonists for excitatory amino acid receptors. Trends Neurosci. 10, 265–272.

Watson, G., Lanthorn, T.H., 1990. Pharmacological characteristics of cyclic homologues of glycine at the. Neuropharmacology 29, 727–730.

Weissman, B.A., Kadar, T., Brandeis, R., Shapira, S., 1992. N^{G}-nitro-L-arginine enhances neuronal death following transient forebrain ischemia in gerbils. Neurosci. Lett. 146, 139–142.

Wong, E.H.F., Kemp, J.A., Priestley, T., Knight, A.R., Woodruff, G.N., Iversen, L.L., 1986. The anticonvulsant MK-801 is a potent *N*-methyl-D-aspartate antagonist. Proc. Natl. Acad. Sci. U.S.A. 83, 7104–7108.

Zebrowska-Lupina, I., 1980. Presynaptic alpha-adrenoceptors and the action of tricyclic antidepressant drugs in behavioural despair in rats. Psychopharmacology 71, 169–172.