Direct Inhibitory Effect of Fluoxetine on N-Methyl-D-Aspartate Receptors in the Central Nervous System

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Background: Data accumulated in the last decade indicate that N-methyl-D-aspartate (NMDA) receptors might be involved in the pathophysiology of depression and the mechanism of action of antidepressants, although a direct inhibitory effect has been reported only in connection with tricyclic compounds, which interact with a wide range of receptors.

Methods: Using whole-cell patch-clamp recording in rat cortical cell cultures, we investigated whether the selective serotonin reuptake inhibitor fluoxetine, which has a much better adverse effect profile, has a direct effect on NMDA receptors, and we compared its action to that of the tricyclic desipramine.

Results: Both desipramine (concentration that causes 50% inhibition (IC_{50}) = 3.13 μM) and fluoxetine (IC_{50} = 10.51 μM) inhibited NMDA-evoked currents with similar efficacy in the clinically relevant low micromolar concentration range. However, in contrast to desipramine, the inhibition by fluoxetine was not voltage-dependent, and fluoxetine partially preserved its ability to associate with NMDA receptor in the presence of Mg^{2+}, suggesting different binding sites for the two drugs.

Conclusions: The fact that different classes of antidepressants were found to be low-affinity NMDA antagonists suggests that direct inhibition of NMDA receptors may contribute to the clinical effects of antidepressants.

Key Words: Antidepressant action, depression, desipramine, fluoxetine, NMDA receptor, whole cell patch clamp

Depression is one of the major psychiatric diseases; its life-time prevalence moves between 10–25% depending on the population investigated (1). In spite of this, the neurochemical background of depression and the mechanism of action of recently used antidepressants is not fully understood (2–5). Accumulating evidence indicates that N-methyl-D-aspartate (NMDA) receptors are involved in the pathophysiology of depression (6,7). NMDA antagonists displayed antidepressant activity in different animal models (8,9) and in clinical studies (10,11). In line with this, preclinical and clinical observations show that antidepressant drugs reduce NMDA receptor function (12–14) although a direct inhibitory effect was demonstrated only with tricyclic compounds (15–17). Members of this class of antidepressants interact with a wide range of receptors (including e.g., muscarinic and nicotinic acetylcholine receptors (AChRs), serotonin (5-HT3) receptors, alpha1-adrenoceptors, histamine H1 receptors), therefore the significance of their effect on NMDA receptors was not recognized.

Previously we have shown that monoamine uptake blocker-type antidepressants (including desipramine, fluoxetine, citalopram and nomifensine) with different chemical structure and selectivity are able to inhibit neuronal nicotinic acetylcholine receptors (nAChRs) in the clinically relevant submicromolar concentration range (18,19). Although structurally different, NMDA receptors share some common pharmacological properties with nAChRs. The channel blocker-type nAChR antagonist mecamylamine is able to block NMDA receptors (20), whereas the channel blocker-type NMDA receptor antagonist MK-801 inhibits nAChRs (21). Our previous data and the literature indicates that the monoamine uptake blocker-type antidepressants inhibit nAChRs by a channel blocker mechanism (22–25), and desipramine inhibits NMDA receptors by an open channel mechanism (15), therefore we hypothesized that NMDA receptor antagonism might be a general attribute of monoamine uptake blocker-type antidepressants.

In the present study our aim was to investigate whether the selective serotonin reuptake inhibitor (SSRI) fluoxetine, which has a much better adverse effect profile than tricyclic drugs, has any effect on NMDA receptors in the central nervous system and to characterize the nature of the possible inhibition. For this purpose we measured the effect of fluoxetine on NMDA-induced currents in whole cell patch clamp experiments in rat cortical cell cultures and compared its efficacy and inhibitory character to that of desipramine.

Methods and Materials

All experimental procedures were approved by the local ethical committee and were in accordance with National Institute of Health (NIH) guidelines (Guide for the Care and Use of Laboratory Animals).

Cortical Neuronal Cultures

Neuronal cultures were prepared for electrophysiology with slight modifications as previously described (26,27). Briefly, pregnant rats (17–18 day gestation) were anesthetized with the mixture of ketamine (50 mg/mL) and xylazine (10 mg/mL). The uterus was dissected out and placed into a laminar airflow box, from this stage the preparation was performed in sterile environment. Individual fetuses were isolated; their whole brains were put into cold minimal essential medium, and kept there during further dissection. Cortex of 4–6 fetuses were dissected out, incubated in .25% trypsin for 10 min, mechanically dissociated in minimal essential medium containing 10% fetal bovine serum, and plated at a density of 150,000–300,000/35 mm Petri dish (precoated with poly-L-lysine, 2 μg/mL). At 24 hour after plating,

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the medium was replaced with B27 supplemented Neurobasal medium (GIBCO, Paisley, United Kingdom), containing 25 μM 2-mercaptoethanol, .5 mM glutamine and 25 μM glutamate. Half of the medium was changed twice a week thereafter to the same (Neurobasal+B27) medium without glutamate. NMDA-evoked currents were recorded from cortical cells (7–21 days in vitro).

Whole Cell Patch-Clamp Recordings in Cortical Neurons

Transmembrane currents were recorded according to the standard whole-cell patch-clamp technique using an Axopatch 200B amplifier and the pClamp software (Axon Instruments, Foster City, California). All experiments were performed at room temperature (22–25°C). Borosilicate glass patch pipettes (1.4–3.8 MΩ) were used. Series resistance was compensated to 60-80%. Pipettes were filled with intracellular solution of the following composition (in mM): CsCl 70, CsF 70, NaCl 10, HEPES 10, Cs-EGTA 10; pH was adjusted to 7.3 with CsOH. The composition of the external solution was (in mM): NaCl 150, KCl 5, $CaCl_2$ 1.4, glucose 10, HEPES 5; tetrodotoxin (TTX) .0003; picrotoxin .1; strychnine .002; glycine .01; pH 7.3 was adjusted with NaOH. Currents were low-pass filtered at 2 kHz using the built-in four-pole low pass Bessel filter of the amplifier and sampled at a rate of 10 kHz.

A pressure-operated, computer-controlled rapid drug application device (DAD-12; Adams and List, Westbury, New York) was used for drug administration. In the bath perfusion protocol NMDA was applied to elicit I_{NMDA} repetitively from a holding potential of –70 mV for 1 sec every 2 min. After two control currents, a certain concentration of the test drugs were applied in bath perfusion for 12 min (6 currents) and then washed out (4 currents). The second control current was considered to be 100%. To determine inhibition, the last I_{NMDA} under the test drug (the 8th current) was expressed as percentage of the 8th control current. When the time course of inhibition was investigated, the nth test current always was compared to the corresponding nth control current.

In the fast drug-administration protocol the inlets of the two glass U-tubes were connected to the pressure control unit of the DAD-12, the outlet to a preistaltic pump, in order to gain precise control of both the inflow and the outflow. Drug application was evoked by closing the electric valve (controlled by the pClamp software) at the outlet of one of the U-tubes. This arrangement allowed rapid application and removal of drugs: 10 to 90% solution exchange times were in the 2 to 10 msec range, as judged by junction potential measurements, and the rate of removal was not dependent on the duration of drug application.

Electric distance values were determined using the equation derived from the special case of the Woodhull model, in which the binding site is assumed to be accessible from the extracellular medium only (28):

$$I_{rel} = K_{(0mV)}/\{K_{(0mV)} + [B] * \exp(-z\delta FE/RT)\}$$

where I_{rel} is the relative amplitude of the current at a presence of a certain blocker concentration [B]; $K_{(0mV)}$ is the dissociation constant at 0 mV, z the valence of the tested drug, δ the electric distance (the fraction of the membrane potential acting on the blocker at its binding site), F Faraday's constant, R the universal gas constant, T the absolute temperature, and E the membrane potential. Electric distance (δ) values are given by fitting this equation to the plot of I_{rel} as a function of E.

Concentration – inhibition curves were fit to the Hill equation:

$$I = I_{control}/[1 + ([D]/IC_{50})^{nH}]$$

where [D] is the drug concentration, IC_{50} is the concentration that causes 50% inhibition, and n_H is the Hill coefficient.

Statistical significance was determined using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; or two-tailed *t* test where appropriate, $p < .05$ was considered significant. Results are presented as mean ± SEM. Voltage dependence was analyzed by the Pearson correlation analysis (GraphPad Instat). Curve fittings were performed either by the pClamp software or by the Solver function of Microsoft Excel.

Materials

Desipramine HCl, fluoxetine HCl, strychnine, glycine, N-methyl-D-aspartate (NMDA) were purchased from Tocris (Tocris Cookson Ltd, Bristol, United Kingdom). Tetrodotoxin and picrotoxin were obtained from Sigma (Taufkirchen, Germany). All other chemicals were of analytical grade.

Results

Effect of Fluoxetine and Desipramine on NMDA-Evoked Currents: Bath Perfusion Protocol

Repeated pressure application of 10 μM NMDA for 1 sec (in a Mg^{2+}-free medium) to 7–21 days old cortical neurons at a holding potential of –70 mV every 2 min, evoked inward currents (Figure 1, upper left recordings). The average amplitude of the NMDA currents was –523.47 ± 40.95 pA ($n = 59$). The amplitude

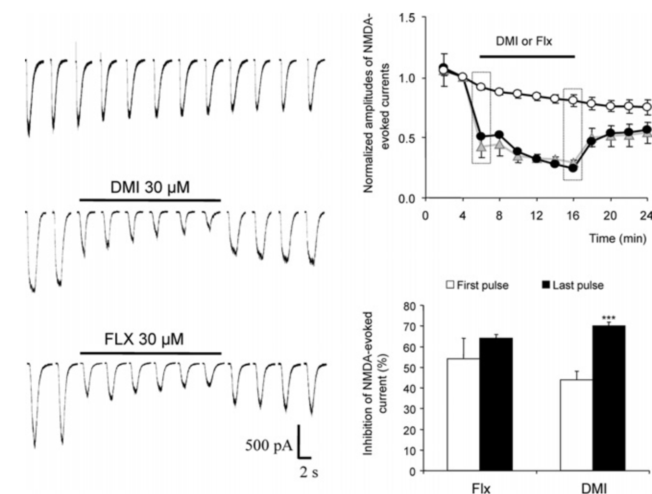

Figure 1. Inhibition of NMDA currents by desipramine and fluoxetine in cultured cortical cells (bath perfusion protocol). In whole cell voltage-clamp experiments NMDA (10 μM) was applied repetitively at a holding potential of –70 mV for 1 sec in every 2 min. The upper recording in the left panel shows control NMDA currents. After two control currents, 30 μM of desipramine (DMI) (left panel, middle recording) or fluoxetine (Flx) (left panel, lower recording) was perfused for 12 min as indicated by the solid line and then washed out. Upper right panel: Normalized NMDA currents (mean ± SEM) and their inhibition by desipramine (30 μM; $n = 6$, filled circles) and fluoxetine (30 μM; $n = 5$, triangles). Control currents are represented by open circles. The second control current was taken as 100% and all subsequent currents were expressed relative to this value. Lower right panel: Time course of inhibition by desipramine and fluoxetine. Bar graph shows the inhibitory effect of antidepressants on the first and last NMDA-induced currents during the 12-min perfusion. Inhibition was calculated by a comparison to the corresponding control value (see dotted squares on the upper right panel), data are the mean ± SEM of 5–6 experiments, two tailed *t*-test was used for statistical comparison, *** $p < .001$). NMDA, N-methyl-D-aspartate.

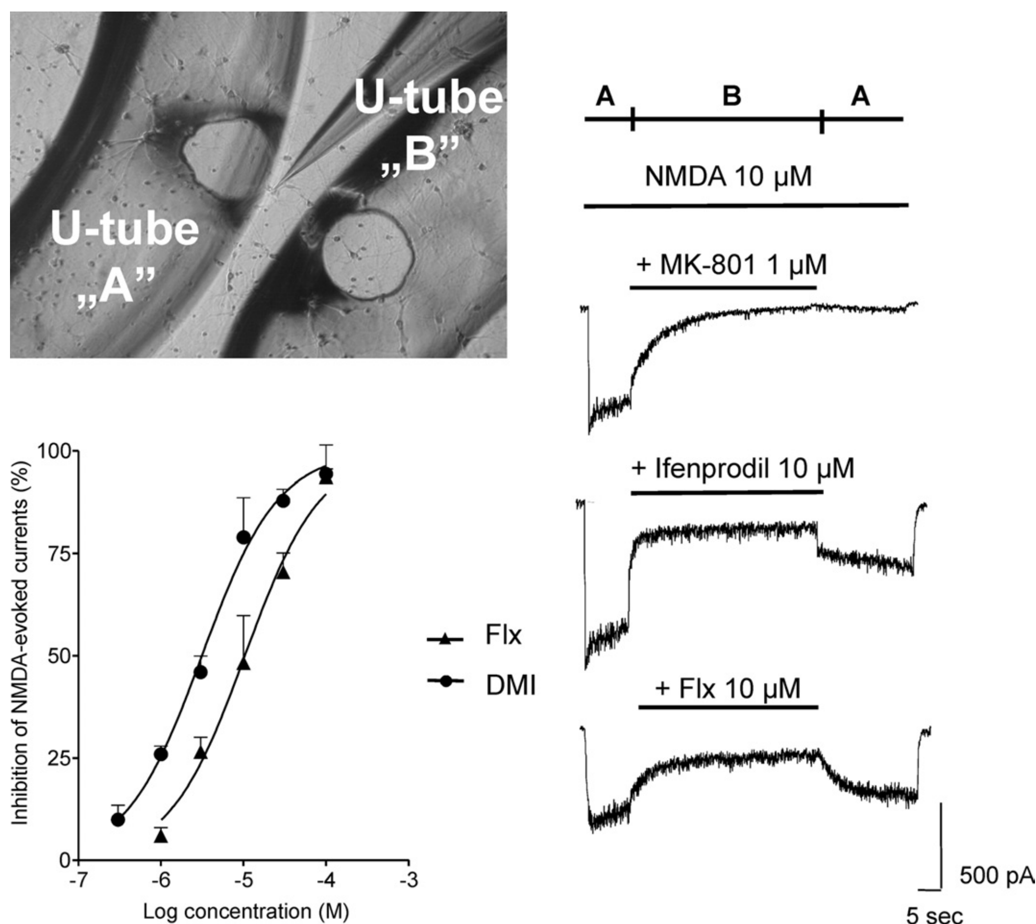

Figure 2. Inhibition of NMDA currents by fluoxetine in cultured cortical cells (fast application protocol). Upper left photo: The solution was changed with two "U-tubes" in the direct environment of the patched cell. Right panel: The cells received NMDA (10 μM) alone for 10 msec ("A"-tube), then NMDA plus antagonists or antidepressants at different concentrations ("B"-tube) for 40 msec, finally NMDA only ("A"-tube) again for 20 msec ("ABA" drug administration protocol, upper panel). Lower panel: representative traces of the effect of the NMDA antagonist MK-801 (1 μM), the NR2B antagonist ifenprodil (10 μM) and fluoxetine (10 μM). Lower left panel: dose-response curve of desipramine (closed circle), and fluoxetine (gray triangle). The IC_{50} values were determined by nonlinear regression (Prism 3.0). NMDA, N-methyl-D-aspartate; $IC_{50,}$ concentration that causes 50% inhibition.

of NMDA-evoked currents decreased to 80.54 ± 4.5% within 12 min, during 6 NMDA pulses (Figure 1, upper right panel).

The initial concentration (30 μM) of drugs was selected on the basis of previous studies in which desipramine inhibited NMDA currents in the 20–50 μM concentration range (15). Perfusion of antidepressants at a concentration of 30 μM for 12 min caused a significant inhibition of NMDA-evoked currents (Figure 1, upper right panel). Although both drugs inhibited NMDA currents with a similar efficacy, the time-course of inhibition was different. The inhibitory effect of desipramine continuously increased. It was 44% at the first NMDA pulse and significantly higher, 70% at the last pulse ($p < .001$, two tailed *t*-test). In contrast, the inhibitory effect of fluoxetine was relatively stable: 54% at the first pulse and 64% at the last NMDA stimulation (Figure 1, lower right panel), the difference between the inhibition of first and the last pulse was not significant. To eliminate the effect of rundown observed in control experiments, inhibition was determined as a percentage of the corresponding control current (see dotted squares on Figure 1, upper right panel).

Effect of Fluoxetine and Desipramine on NMDA-Evoked Currents: Pressure Application Protocol

To study the differences in the action of fluoxetine and desipramine in more detail, a fast drug application system was used in further experiments. Two glass 'U-tubes' were placed close to the patched cell (Figure 2, upper left photo), and the following drug administration protocol was applied: NMDA for 10 sec (A-tube), antagonists, fluoxetine or desipramine co-applied with NMDA for 40 sec (B-tube), NMDA for 20 sec (A-tube). Under these conditions, the offset rate of NMDA currents without antagonists or antidepressants was 284 ± 15 msec, and the response to NMDA was almost completely blocked (97.57 ± .94% inhibition, $n = 5$) by the NMDA antagonist MK-801 (1 μM) and very significantly blocked (76.05 ± 6.05, $n = 9$) by the selective NR2B antagonist ifenprodil (10 μM), suggesting that in the cultured cortical cells the response to NMDA is mediated primarily by NR2B subunit containing NMDA receptors. The antidepressants dose-dependently inhibited the NMDA-evoked inward currents. The IC_{50} values were in the low micromolar range; for desipramine and fluoxetine 3.13 μM (n_H = .95) and 10.51 μM (n_H = 1.02), respectively (Figure 2, lower left panel).

Voltage Dependence of the Inhibition of NMDA Currents by Fluoxetine and Desipramine

In order to investigate the mechanism of inhibition, the voltage dependence of the effect of antidepressants (30 μM

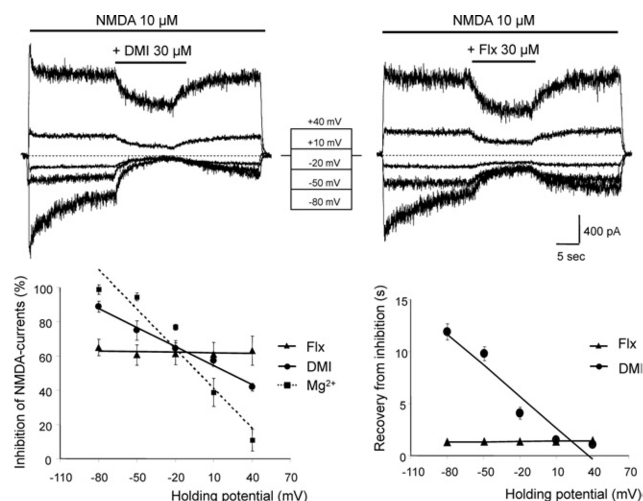

Figure 3. Voltage-dependence of the inhibition of NMDA currents and the recovery from inhibition by desipramine and fluoxetine. "ABA" drug administration was used at different holding potentials (ranging from −80 mV to +40 mV, see inset). The whole sequence was performed on the same cell. The 0 current level is represented by dotted lines. Upper left recording: Representative currents for desipramine. Upper right recording: Representative currents for fluoxetine. Lower left panel: Inhibition of NMDA-currents as a function of membrane potential. Each point represents the mean ± SEM of 5–6 independent experiments. Data were analyzed using Pearson correlation analysis. The dotted line represents the voltage dependence of Mg^{2+}. Lower right panel: recovery from inhibition by desipramine and fluoxetine as a function of holding potential. The rates of recovery from inhibition (τ_{offset}) were determined by exponential curve fit. Data are mean ± SEM of 5–6 independent experiments. Voltage-dependence was determined by Pearson correlation analysis. NMDA, N-methyl-D-aspartate.

each) was studied. We made recordings at −80, −50, −20, +10, +40 mV, using the same drug administration sequence, as described in Figure 2, with modified durations because of the faster onset of inhibition at 30 μM (15 sec, 10 sec, 15 sec). Magnesium (Mg^{2+}) is a well-known open channel blocker of the NMDA receptor. The inhibition of NMDA-evoked currents by Mg^{2+} (5 mM) was strongly voltage-dependent (Figure 3, lower left panel). The inhibition by desipramine (30 μM) was also obviously voltage-dependent (Figure 3, upper and lower left panels), being almost complete at −80 mV, and decreasing with depolarization of the membrane. A significant inhibition (43%) was still observable even at +40 mV. The slope of inhibition versus voltage plot was significantly different from zero (Pearson correlation coefficient: $r^2 = .98$, $p < .001$, $n = 6$). In contrast, the inhibition of NMDA-currents by fluoxetine (30 μM) was not dependent on the membrane potential (Figure 3, upper right and lower left panels). The slope was not significantly different from zero ($r^2 = .004$, $p = .63$, $n = 6$). Both at negative and positive membrane potentials, the degree of inhibition was almost constant, ranging from 61 to 65% (Figure 3, lower left panel).

Estimated Location of the Binding Sites by the Woodhull-Model

According to the Woodhull-model, the voltage dependence of inhibition correlates with the location of the binding site within the electric field of the membrane (28). The electric distance (δ) for fluoxetine, desipramine and Mg^{2+} was calculated from the Woodhull-equation. For Mg^{2+} the δ value was .71 under our experimental conditions, which is in line with previous data from the literature and indicates a deep binding site within the pore of NMDA receptor. The calculated δ was .44 for desipramine and .01 for fluoxetine, indicating that the hypothetic binding site of these two antidepressants is most probably different and fluoxetine does not need to overcome the electric field to access its binding site.

Voltage Dependence of the Rate of Recovery from Inhibition

To further study the differences, the rate of recovery from inhibition (τ_{offset}) was also investigated. Exponential equations were fit to the decay phase, and the time constants were plotted as a function of the holding potential (−80, −50, −20, +10, or +40 mV) (Figure 3, lower right panel). In the presence of desipramine (30 μM) depolarization accelerated the recovery from inhibition. This acceleration was strictly voltage dependent, the slope was significantly different from zero ($r^2 = .68$, $p < .001$). In contrast, the recovery from inhibition by fluoxetine (30 μM), was constant, not dependent on the holding potential. The slope was not significantly different from zero ($r^2 = .93$, $p > .001$).

Effect of Mg^{2+} on the Interaction of Fluoxetine and Desipramine with the NMDA Receptor

Since Mg^{2+} inhibits NMDA-receptors through an open-channel blocker type mechanism, the question arises, whether fluoxetine or desipramine can associate with the NMDA-receptor in the presence of Mg^{2+}. We compared, therefore, the rate of recovery from inhibition (τ_{offset}) of both antidepressants (30 μM) in the absence and presence of Mg^{2+} (Figure 4). The recovery from inhibition by Mg^{2+} (5 mM) alone was very fast (τ_{offset} = 459 ± 80 msec at −70 mV). The offset rate of inhibition by desipramine alone (τ_{offset} = 10,461 ± 588 msec) was significantly slower than that of fluoxetine alone (τ_{offset} = 3437 ± 1016 msec) (Figure 4, right panels). Application of desipramine in the presence of Mg^{2+} (τ_3 = 507 ± 17 msec) did not change the rate of recovery from inhibition by Mg^{2+} alone, indicating that desipramine is not able to associate with the NMDA receptors in the presence of Mg^{2+} (Figure 4, left panels). In contrast, fluoxetine significantly affected the recovery rate of inhibition by Mg^{2+}: the recovery from inhibition by co-applied Mg^{2+} and fluoxetine (τ_{offset} = 1111 ± 251 msec) was slower than recovery from inhibition by Mg^{2+}, but faster than recovery from inhibition by fluoxetine alone (Figure 4, lower right panel).

Discussion

Inhibitory Effect of Fluoxetine on NMDA Receptors

The major question of our study was whether the SSRI fluoxetine has any direct effect on NMDA receptors in the central nervous system. We used desipramine as a reference compound, because previously it has been reported that this tricyclic antidepressant blocks NMDA receptors with an open channel mechanism in the 20–50 μM concentration range (15), and several subsequent studies confirmed this action using different experimental approaches (16,17,29,30). We found that fluoxetine inhibits NMDA-currents in cortical cells in the low micromolar concentration range with a similar efficacy as desipramine. Is there a clinical significance of this finding? During antidepressant therapy the plasma concentration of these drugs is about 1–2 μM (31,32), and even higher concentration can be observed in the brain. In a magnetic resonance spectroscopy study the steady-state brain concentration of fluoxetine was found to be 13 ± 6 μM in depressed patients (33). A similar accumulation was reported in a study which investigated the pharmacokinetic properties of desipramine (34). These data and our results suggest that both the SSRI fluoxetine and the tricyclic desipra-

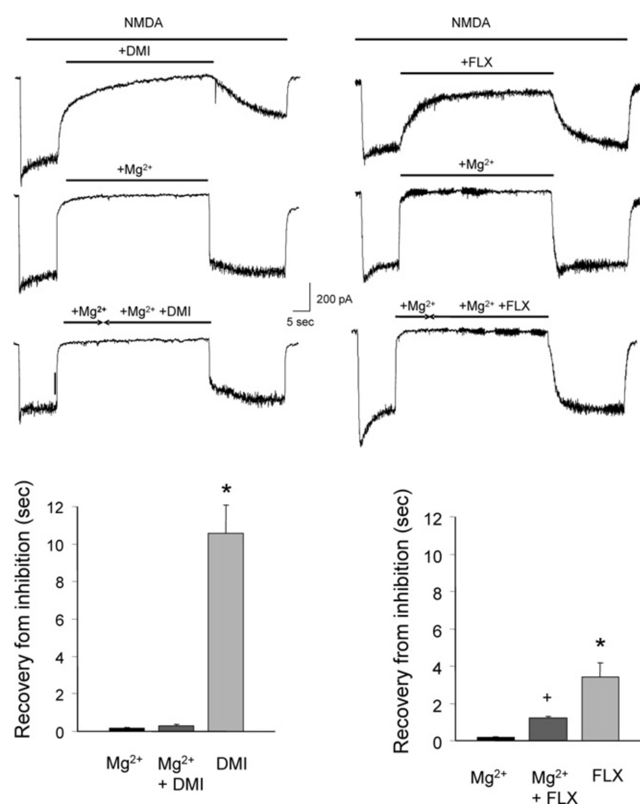

Figure 4. Inhibition of NMDA currents by fluoxetine and desipramine in the presence of Mg^{2+}. After a 10-sec NMDA (10 μM) application, Mg^{2+} (5 mM) or the antidepressants desipramine (DMI) or fluoxetine (Flx) were applied at a concentration of 30 μM for 40 sec in the presence of NMDA, then NMDA was applied alone for an additional 20 sec to investigate the recovery from inhibition. When the interaction of Mg^{2+} and antidepressants was investigated, the application of antidepressants started 10 sec after the beginning of Mg^{2+} application as shown by the horizontal bars (Upper left recordings: desipramine, upper right recordings: fluoxetine). The rates of recovery from inhibition (τ_{offset}) were determined by exponential curve fit. Data are the mean ± SEM of 4–6 independent experiments (lower left panel: desipramine, lower right panel: fluoxetine). Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test, * $p < .05$ compared to Mg^{2+} alone, $^{+}$ $p < .05$ compared to Mg^{2+} alone. NMDA, N-methyl-D-aspartate.

mine are able to inhibit NMDA receptors during antidepressant treatment. Since accumulating evidence indicates that modulation of NMDA receptor function might be beneficial in the treatment of depression (10,11,49), it is reasonable to assume that this action of antidepressants might contribute to their therapeutic effect.

Although the investigation of a possible NMDA receptor subtype selectivity of antidepressants was not our aim, the kinetic analysis of NMDA response (35) and the use of the selective NR2B antagonist ifenprodil (36) suggest that in our preparation the NMDA-induced current is mediated predominantly via NR2B containing NMDA receptors. The fact that fluoxetine and desipramine effectively block these receptors supports our conclusion on the possible antidepressant effect of NMDA antagonism. According to the network hypothesis of depression, reduction of the number of neurons and/or synaptic connections due to different harmful events (e.g., stress-induced reduction of neurotrophic support) might lead to the development of depression and reversal of these processes through enhancing plasticity and cellular resilience results in antidepressant effect (37,38). It has been shown that NR2B receptors are mainly extrasynaptic, and their stimulation might lead to apoptotic cell death (39,40), whereas NR2A receptors are located primarily in synapses and their physiological activation results in plastic changes and upregulation of neurotrophic factors like BDNF (41). Thus the inhibition of NR2B receptors by antidepressants might help the recovery of neuronal networks by preventing neurotoxic cell damage (42). Based on our results we can conclude that NR2B receptors are certainly blocked by antidepressants but the sensitivity of NR2A receptors to antidepressants is unknown. Systematic clarification of a possible subtype selectivity is beyond the scope of this paper and requires further study.

Different Sites of Action of Fluoxetine and Desipramine on NMDA Receptors

In the bath perfusion experiments we observed an important difference in the action of the two antidepressants: the inhibitory effect of desipramine continuously increased with time, whereas the effect of fluoxetine was less dependent on the duration of perfusion (Figure 1, lower right panel). In order to analyze the differences we used a fast drug-application system, which made it possible to compare the kinetics of inhibition. In these experiments the NMDA receptor-mediated currents were blocked by desipramine in a voltage-dependent manner; the inhibition was attenuated by depolarization. In contrast, the inhibition by fluoxetine showed no voltage-dependence. This difference suggests that the inhibitory effect of desipramine is dependent on the activity state of neurons and becomes weaker if the cells are depolarized, whereas fluoxetine has the same inhibitory effect in the whole spectrum of membrane potentials, that is, its action is not influenced by the activity pattern of neurons. Nevertheless, this dissimilarity does not appear in practice, since the clinical efficacy of fluoxetine and desipramine is not significantly different (43).

According to the Woodhull-model (28) the voltage-dependence of inhibition by a drug provides a good approximation of the depth of binding in the transmembrane electric field. This model is used to estimate the electric distance (δ): the fraction of the electric field sensed by a charged antagonist entering the ion channel. The calculated electric distance was .44 for desipramine, and .01 for fluoxetine, which predicts a deeper binding site for desipramine within the transmembrane electric field and a shallower binding site for fluoxetine, probably close to the extracellular surface of the receptor. In line with this, depolarization accelerated the dissociation of desipramine from NMDA receptor, but had no effect on the dissociation rate of fluoxetine (Figure 3, lower right panel).

The different site of interaction is also supported by the different ability of the two drugs to interfere with the NMDA receptor in the presence of Mg^{2+}. Desipramine did not change the rate of recovery from inhibition by Mg^{2+} suggesting that desipramine was not able to associate with the NMDA-receptor channel in the presence of Mg^{2+} (Figure 4, left panels). Our finding that the calculated electric distance of Mg^{2+} and desipramine were different (.71 vs. .44), indicates that desipramine and Mg^{2+} does not bind to the same location, but the binding of Mg^{2+} initiates a conformational change in the three dimensional structure of NMDA-receptors, which prevents the association of desipramine. In contrast to desipramine, the recovery rate from inhibition by fluoxetine in the presence of Mg^{2+} was significantly lower than the recovery rate from inhibition by Mg^{2+} alone. This result suggests that fluoxetine is partially able to associate with

the NMDA-receptor even in the presence of Mg^{2+}, which also supports a shallower localization of binding site for fluoxetine. Nevertheless, an interaction between the binding of Mg^{2+} and fluoxetine exists, because the recovery from inhibition by fluoxetine alone was much longer, which suggests that the presence of Mg^{2+} significantly hinders the association of fluoxetine to the NMDA receptor. In summary, our data indicate that although both antidepressants inhibit NMDA receptors with similar efficacy, the mechanism of action of the two drugs are not identical.

A Possible Role of NMDA Receptors in the Pathophysiology of Depression and in the Action of Antidepressants

Converging lines of evidence indicates the possible role of glutamatergic neurotransmission, and more specifically NMDA receptors in the pathophysiology of depression (12,14). The antidepressant-like effect of competitive and noncompetitive NMDA receptor antagonists has been shown in several behavioral experiments (8,9,44–47). Co-administration of the noncompetitive NMDA antagonists amantadine and memantine with fluoxetine, venlafaxine or imipramine resulted in synergistic antidepressive effect in forced swimming test of rats (48). Important clinical observations also support the involvement of NMDA receptors in the antidepressant action. In preliminary clinical studies amantadine improved the depressive score in treatment-resistant depression (49). In two placebo-controlled double-blind studies on patients with major depression, infusion of the NMDA antagonist ketamine hydrochloride resulted in significant improvement within 72 hours (10) or within 110 min, and the beneficial effect remained significant for at least one week (11). These data indicate that the function of NMDA receptors is linked to the neurochemical events of depression.

This idea is supported by observations, which show that NMDA receptors might also be involved in the mechanism of action of antidepressants. Numerous biochemical (50), electrophysiological (51) and behavioral (52) studies have confirmed the reduced NMDA receptor function during chronic antidepressant treatment. Both classic and atypical antidepressants were able to attenuate NMDA receptor function indirectly (14,53). Chronic but not acute antidepressant treatment caused decreased NMDA receptor function as indicated by reduced radioligand binding (12), suggesting an involvement of NMDA receptors in the altered plasticity during antidepressant therapy. Several possible mechanisms have been suggested to explain the association between antidepressant treatment and the modulation of NMDA receptor function. Most studies proposed indirect mechanisms, like a reduction in glutamate release by different synaptic and nonsynaptic mechanisms (2,54–57,58), modulation of Zn^{2+} concentration in several key regions of the brain (59), or the regulation of BDNF expression (60,61). A direct action has only been reported in connection with tricyclic compounds (15–17). Our data, that a representative of SSRI compounds, which has much less adverse effects than the tricyclic antidepressants, has the ability to inhibit NMDA receptors in the clinically relevant concentration range, provide an important support for the idea that glutamatergic neurotransmission and modulation of NMDA receptor function are important in the treatment of depression, and inhibition of NMDA receptors might be a general property of antidepressants.

In conclusion, our results indicate that fluoxetine and desipramine inhibit NMDA receptors in the clinically relevant concentration range with a similar efficacy but with different mechanism of action. This effect has important human pharmacological implications because direct modulation of NMDA receptor function might be pivotal in development of the therapeutic action of antidepressants.

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