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Effect of MK-801 and ketamine on hydroxyl radical generation in the posterior cingulate and retrosplenial cortex of free-moving mice, as determined by in vivo microdialysis

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Abstract

This study investigated the effect of MK-801 and ketamine, *N*-methyl-D-aspartate (NMDA) receptor antagonists which can induce schizophrenic symptoms and have neurotoxicity in human and animals, on hydroxyl radical (·OH) generation in the posterior cingulate and retrosplenial (PC/RS) cortex of free-moving mice using the salicylic acid trapping technique. MK-801 (0.6 mg/kg) or ketamine (50 mg/kg) acute administration significantly increased ·OH levels in mouse PC/RS cortex. The basal ·OH levels after MK-801 and ketamine administrations for 7 consecutive days were significantly increased compared with the naive basal levels. MK-801 (0.6 mg/kg) or ketamine (50 mg/kg) challenge after chronic administration further significantly increased dialysate levels of ·OH. Our study also found that the release of ·OH was secondary to stereotyped behavior, and the intensity of stereotyped behavior induced by MK-801 was more than that induced by ketamine. The results suggested that NMDA receptor antagonists participate in the generation of ·OH in the PC/RS cortex of mouse, and oxidative stress, derived from the formation of free radicals, might play an important role in the pathophysiology of these two models of schizophrenia.
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Keywords: MK-801; Ketamine; Posterior cingulate and retrosplenial cortex; Hydroxyl radical; Microdialysis; Schizophrenia

1. Introduction

At present, the etiology of schizophrenia still remains elusive. Non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists, such as phencyclidine (PCP), ketamine and (+)-MK-801 (dizocilpine), have psychotomimetic effects in normal individuals (Duncan et al., 1999; Krystal et al., 1994) and exacerbate pre-existing symptoms in schizophrenic patients (Duncan et al., 1999; Lahti et al., 1995). In animals, they also evoke locomotor hyperactivity, stereotypy and sniffing behavior (Koek et al., 1988). The psychosis induced by NMDA receptor antagonists has derived considerable scientific interest since it represents the probably most accurate pharmacological model of schizophrenia available (Moghaddam and Jackson, 2003).

NMDA receptor antagonists induced hypofunction has been shown to affect multiple corticolimbic brain regions. Among these regions, the posterior cingulate and retrosplenial (PC/RS) cortex appears to be most susceptible (Lahti et al., 1995; Olney et al., 1991; Tendolkar et al., 2004). Studies in rats and mice demonstrate neurotoxicity such as structural damage, reversible or irreversible neuronal alterations, neural disinhibition, and c-fos or heat shock protein expression particularly in the PC/RS cortex during systemic administration of NMDA receptor antagonists like MK-801, ketamine and PCP (Brosnan-Watters et al., 1999; Li et al., 2002; Nakki et al., 1996; Nishizawa et al., 2000; Olney et al., 1991, 1999; Sharp et al., 1991). However, the mechanism for neurotoxicity of NMDA receptor antagonists and the subsequent degenerative process in the PC/RS cortex

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have not fully been understood. The study by Rajdev et al. (1998) suggested that PCP induced injury in the PC/RS cortex was associated with increased oxidative stress and antioxidants could block this injury.

Oxidative stress refers to an imbalance between the formation of cellular oxidants and the antioxidative processes and it occurs when the production of reactive oxygen radicals (ROS) (or free radicals) such as the superoxide anion ($\cdot O_2^-$), hydroxyl radical (·OH) and hydrogen peroxide (H_2O_2) increase beyond the physiological antioxidant protective mechanisms and is associated with numerous pathological states, including diseases of the nervous system (Gutteridge and Halliwell, 2000; Schulz et al., 2000). Among the ROS, ·OH that derives from three electron reduction of O_2 is considered to be the most devastatingly reactive free radical, and it can attack on molecules near where it is formed. The possibility that free radicals contribute to schizophrenic pathophysiology was first proposed in 1954. Subsequently there is increasing evidence that free radical-mediated CNS neuronal dysfunction is involved in the pathophysiology of schizophrenia (Do et al., 2000; Mahadik and Mukherjee, 1996; Yao et al., 2001). When free radicals are generated in excess or the cellular antioxidant defense system is deficient, they can stimulate chain reactions by interacting with proteins and nucleic acids and causing cellular dysfunction and even death, which further deteriorates schizophrenic symptoms (Mahadik and Mukherjee, 1996).

In light of the close relationship between oxidative stress and schizophrenia, it is necessary to know whether the ·OH is altered in the PC/RS cortex of schizophrenic animal models induced by NMDA receptor antagonists. If so, it would provide further evidence that oxidative stress, derived from the formation of free radicals, may be involved in NMDA receptor antagonists-induced neurotoxicity in the PC/RS cortex and there exists a substantial risk of free-radical mediated neuronal damage in schizophrenia. In addition, schizophrenia is a chronic illness, and psychotic symptoms resulting from single-dose infusions of ketamine in normal subjects tend to be mild and somewhat inconsistent; in contrast, prolonged exposure in PCP abusers is associated with severe, persistent psychotic symptoms (Tsai and Coyle, 2002). Our previous study also suggests that there are different effects of acute and chronic MK-801 administrations on the release of glutamate and ascorbic acid (Zuo et al., 2006). Therefore, we conducted an exploratory microdialysis study using MK-801 and ketamine to investigate the effects of single or repeated administration in the PC/RS cortex, a cortex that has received relatively little attention with regard to microdialysis, on extracellular level of ·OH in the mice. At the same time, to estimate the relationship between microdialysis data and behavioral effects of drugs, the pattern of stereotyped behavior was assessed in parallel.

2. Methods

2.1. Animals and surgery

Male Swiss mice, weighing 25–30 g, were used throughout the study. The animals were supplied by the Experimental Animal Centre of Shenyang Pharmaceutical University. Mice were maintained under standard housing conditions in 12L:12D light/dark cycle (light on 6:30) with free access to water and standard food. All procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. The total number of animals as well as their suffering was minimized whenever possible. The procedure to prepare the transversal microdialysis probe was described elsewhere (Wu et al., 1988). The internal diameter of the probe was 200 μm and the external diameter of the probe was 310 μm. The dialysis fiber sampling the PC/RS extracellular space was 2 mm in length and the molecular weight cutoff is 50,000. The mice were anesthetized with chloral hydrate 350 mg/kg (i.p.) and mounted in a stereotaxic frame. The head skin was cut and holes were drilled. The dialysis probe was insert transversally through the PC/RS cortex (anteroposterior, −2.1 mm; mediolateral, ±2.5 mm, Fig. 1). After surgery, the animals were individually housed in a plastic cage and left for recovery about 18–24 h before used for the experiment.

2.2. Drugs

Ketamine and (+)-MK-801 ((5*R*, 10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*, *d*)cyclohepten-5,10-imine hydrogen maleate) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). They were dissolved in 0.9% saline and i.p. in a volume of 0.1 ml/10 g body wt. Controls received 0.9% saline alone.

2.3. Experimental protocol

The animals were randomly divided into control group, MK-801 group and ketamine group. In the chronic treatment experiment, mice in the control, MK-801 and ketamine groups were treated with 0.9% saline, MK-801 (0.6 mg/kg, i.p.) or ketamine (50 mg/kg, i.p.) daily for 7 days, respectively. The dialysis experiment was performed on the eighth day. On

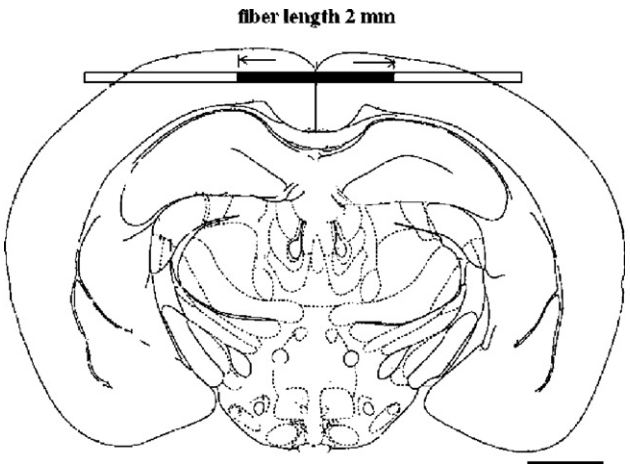

Fig. 1. Placement of microdialysis probe in the PC/RS cortex of mice. The black part represents the active microdialysis membrane of the probe (anteroposterior, −2.1 mm; mediolateral, ±2.5 mm). Scale bar=1 mm.

the day of experiment, Ringer's solution (140 mM NaCl, 3.35 mM KCl, 1.26 mM $CaCl_2$, 1.15 mM $MgCl_2$, 1.35 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, pH 7.4) containing 2 mM sodium salicylate as ·OH trapping reagent was perfused through the dialysis fiber at a constant rate of 4 μl/min. The dialysate during the first 60 min was discard and then the sample was collected every 20 min. Saline, MK-801 (0.6 mg/kg, i.p.) or ketamine (50 mg/kg, i.p.) was administered in the acute experiment or challenged in the chronic experiment when the output of the value became stable in the last three samples with variance less than 10%. When the experiment was finished, the mice were given an overdose of chloral hydrate and their brains were removed from the skull and immersed in 4% paraformaldehyde. Coronal sections (50 μm thick) were cut for checking the position of the dialysis probes. The data were discarded if the probe was incorrectly positioned.

2.4. Determination of ·OH

·OH generation was determined by measuring the extracellular level of 2,3-dihydroxybenzoic acid (2,3-DHBA), which is produced through non-enzymatic hydroxylation of sodium salicylate by ·OH. The extracellular concentration of 2,3-DHBA (20 μl) was measured by high performance liquid chromatography with electrochemical detection (HPLC-ECD) system (Eicom Co., Japan). A reversed-phase column (Eicom-Pack, ODS, 4.6×150 mm) was used and the mobile phase composed of 0.03 M citrate, 0.03 M acetate and 10% methanol. The column temperature was kept at 23 °C and applied potential was set at +700 mV (versus an Ag/AgCl reference electrode). The current produced was monitored by a chromatography workshop (JiangShen Co., China).

2.5. Behavioral observations

In order to investigate the change of behavior after acute and chronic administration of NMDA receptor antagonists, stereotyped behavior was concomitantly measured during the microdialysis experiments. The cage was a clear cubic arena and 40 cm×30 cm×20 cm in size. A video tape recorder above the cage was used to record the animal's behavior. At the end of the test, a trained observer, who was unaware of treatments, rated the stereotyped behavior for 1 min in the middle of microdialysis. Stereotypy rating was performed using the method of Huang et al. (1997): Score 1, lying down, eyes closed (asleep). Score 2, lying down, eyes open (inactive). Score 3, normal grooming or chewing cage litter (in place activities). Score 4, moving about cage, sniffing, rearing (normal, alert, active). Score 5, running movement (hyperactive). Score 6, repetitive exploration of the cage at normal level of activity (slow patterned). Score 7, repetitive exploration of the cage with hyperactivity (fast patterned). Score 8, remaining in same place in cage with fast repetitive head and/or foreleg movement (restricted). Score 9, backing up, jumping, seizures, abnormally maintained postures, dyskinetic movements (dyskinetic-reactive).

2.6. Statistical analysis

Microdialysis data were expressed as percentage of basal values (calculated as means of the three samples before injections). The basal concentrations in the dialysis were expressed in ng/20 μl and given as mean±standard error of the mean (S.E.M.). Data were not corrected for 'recovery' of the dialysis procedure. Two-factor ANOVA for repeated measurements and LSD post hoc test were used to examine the possibility of significant differences ($p<0.05$) between groups followed by a Student's *t*-test for every time-point.

Behavioral data were presented as mean±S.E.M and the statistical method was similar to microdialysis experiment.

3. Results

3.1. Extracellular basal levels of 2,3-DHBA in the PC/RS cortex

Basal extracellular 2,3-DHBA levels of the six groups are given in Table 1. From the table we can see there was no significant difference between naive (i.e., before MK-801, ketamine or saline acute administration) mice and saline administration for 7 consecutive days mice. However, the basal 2,3-DHBA levels before MK-801 and ketamine challenge on the 8th day after 7 consecutive days administration were significantly increased ($p<0.001$ and $p=0.002$, respectively) compared with the naive group.

3.2. Effects of acute systemic administration MK-801 or ketamine on extracellular 2,3-DHBA levels in the PC/RS cortex of mice

Acute administration of MK-801 (0.6 mg/kg, $n=8$) significantly increased dialysate levels of 2,3-DHBA (Fig. 2; for the 20–200 min samples, $F(1,14)=12.601$, $p=0.003$). The maximal increase of 2,3-DHBA was 149.33% after 120 min of drug administration and remained elevated above baseline at the termination of the experiment. Similarly, the release of 2,3-DHBA also began to increase immediately after ketamine (50 mg/kg, $n=8$) administration (Fig. 2; for the 20–200 min samples, $F(1,14)=50.618$, $p<0.001$) and became maximal (150.61%) within 80 min, with a duration of approximately 200 min. Similar increase of MK-801 paralleled those of

Table 1
The 2,3-DHBA basal levels in dialysates from PC/RS cortex before acute administration MK-801 and ketamine or after chronic systemic administration for 7 consecutive days

Drug administration	2,3-DHBA (pg/20 μl)
Saline×1 day	164.18±10.07
MK-801 0.6 mg/kg×1 day	159.39±8.05
Ketamine 50 mg/kg×1 day	166.75±7.69
Saline×7 days	178.67±5.33
MK-801 0.6 mg/kg×7 days	220.14±9.63
Ketamine 50 mg/kg×7 days	262.84±20.08

Baseline was defined as the mean of the three baseline samples prior to the onset of treatment. Data are shown as the mean±S.E.M. ($n=8$–9).

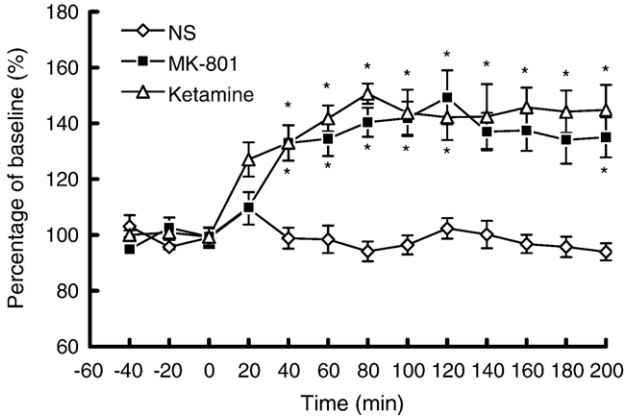

Fig. 2. Effect of acute administrations of MK-801 (0.6 mg/kg, i.p.) or ketamine (50 mg/kg, i.p.) on extracellular 2,3-DHBA levels in the PC/RS cortex of mice. Data are expressed as the percentage of basal levels and are given as mean± S.E.M. (n=8). *p<0.05 compared with the corresponding vehicle control group. Between the vehicle group and the MK-801 group, F(1,14)=12.601, p=0.003, between the vehicle group and the ketamine group, F(1,14)=50.618, p<0.001 (two-way ANOVA for repeated measurements).

ketamine but appeared to lag behind ketamine with a 40 min delay to the maximal.

3.3. Effects of chronic systemic administration MK-801 or ketamine on extracellular 2,3-DHBA levels in the PC/RS cortex of mice

After repeated administration (0.6 mg/kg, n=9) for 7 days, MK-801 (0.6 mg/kg) challenge significantly increased dialysate levels of 2,3-DHBA (Fig. 3; for the 20–200 min samples, F(1,14)=74.028, p<0.001). The maximal increase was 153.99% after 120 min of drug administration. Ketamine challenge also significantly increased dialysate levels of 2,3-DHBA (Fig. 3; for the 20–200 min samples, F(1,14)=25.272, p<0.001) after repeated administration (50 mg/kg, n=9) for 7 days and became maximal (175.61%) within 120 min. From this figure we can see that dialysate levels of 2,3-DHBA induced by MK-801 and ketamine were remained elevated above baseline at the termination of the experiment.

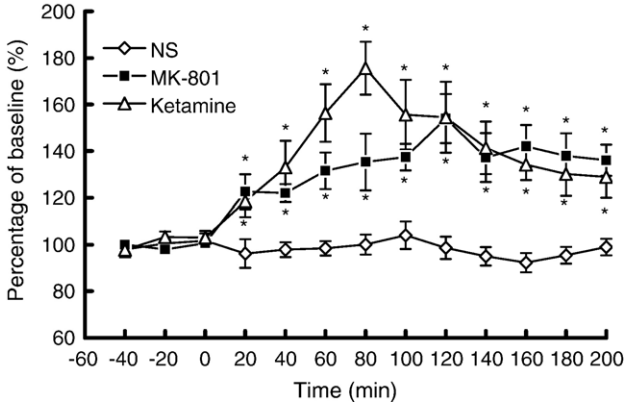

Fig. 3. Effect of MK-801 (0.6 mg/kg i.p.), ketamine (50 mg/kg i.p.) or saline challenge on extracellular 2,3-DHBA levels in the PC/RS cortex of mice treated with MK-801 at 0.6 mg/kg, ketamine at 50 mg/kg, i.p. or saline per day respectively for 7 consecutive days. Data are expressed as the percentage of basal levels and are given as mean± S.E.M. (n=9). *p<0.05 compared with the corresponding vehicle group. Between the vehicle group and the MK-801 group, F(1,14)=74.028, p<0.001, between the vehicle group and the ketamine group, F(1,14)=25.272, p<0.001 (two-way ANOVA for repeated measurements).

3.4. Effects of acute systemic administration MK-801 or ketamine on stereotyped behavior of mice

Both MK-801 (0.6 mg/kg, n=8) and ketamine (50 mg/kg) acute administration induced a characteristic behavioral response with a remarkable hyperactivity. All behavioral effects occurred a few minutes after administration. The stereotypy scores of MK-801 group significantly different from those of control group (Fig. 4; for the 10–190 min samples, F(1,14)=201.625, p<0.001) and reached a maximum at 30 min. Acute administration of ketamine (50 mg/kg, n=8) also significantly increased the scores of stereotypy (Fig. 4; for the 10–190 min samples, F(1,14)=16.106, p<0.001). The maximal increase was at 10 min after drug administration. The maximal increase of ·OH lagged behind that of stereotyped behavior in all mice.

3.5. Effects of chronic systemic administration MK-801 or ketamine on stereotyped behavior of mice

After chronic administration (0.6 mg/kg, 7 days, n=9), MK-801 (0.6 mg/kg) challenge significantly increased the stereotyped scores of mice (Fig. 5; for the 10–190 min samples, F(1,14)=193.870, p<0.001). The maximal increase was also at 30 min after drug administration and the increase duration was more than that of acute MK-801 administration. Ketamine (50 mg/kg) challenge after chronic administration also significantly increased the stereotypy scores of mice (Fig. 5; for the 10–190 min samples, F(1,14)=27.172, p<0.001) and reached a

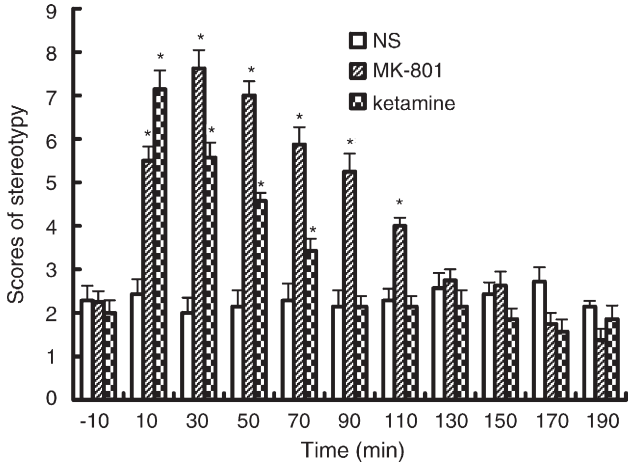

Fig. 4. Effect of acute administration of MK-801 (0.6 mg/kg, i.p.) or ketamine (50 mg/kg, i.p.) on stereotyped behavior of mice. Data are expressed as mean± S.E.M. (n=8). *p<0.05 compared with the corresponding vehicle group. Between the vehicle group and the MK-801 group, F(1,14)=201.625, p<0.001, between the vehicle group and the ketamine group, F(1,14)=16.106, p<0.001 (two-way ANOVA for repeated measurements).

maximum at 10 min with longer increase duration than acute administration. The maximal increase of ·OH was secondary to that of stereotyped behavior in all mice.

4. Discussion

The present study investigated the effects of acute and chronic administration of the non-competitive NMDA receptor antagonists MK-801 and ketamine on extracellular ·OH levels in the PC/RS cortex and the stereotyped behavior of free moving mice. We observed a massive ·OH production during MK-801 or ketamine acute administration. Our additional experiment suggested that the release of ·OH returned to naive baseline level after 320 min of acute administration of MK-801 (data were not shown). That is to say, the ·OH release after acute administration was not permanent. However, after repeated administration, the release of ·OH lasted for a longer time, and the ·OH level was still elevated even after 24 h after the 7th administration, i.e., the basal ·OH levels before MK-801 or ketamine challenge on the 8th day were significantly increased compared with the naive basal level. MK-801 or ketamine challenge after 7 consecutive days administration further significantly increased dialysate levels of ·OH.

NMDA receptor antagonists have been described as neuroprotectant in the therapy of excitotoxicity associated with ischemia and neurotrauma (Gill et al., 1988; McNamara et al., 1988). However, the investigation of Olney's laboratory suggested that acute administration of PCP, MK-801 and ketamine produces a long-lasting, perhaps irreversible necrotic toxicity (Olney et al., 1989), which has sharply curtailed the development of NMDA antagonists as therapeutic agents. Until now, the NMDA receptor antagonists induced psychosis has derived considerable scientific interest since it represents the probably most accurate pharmacological model of schizophrenia available (Moghaddam and Jackson, 2003).

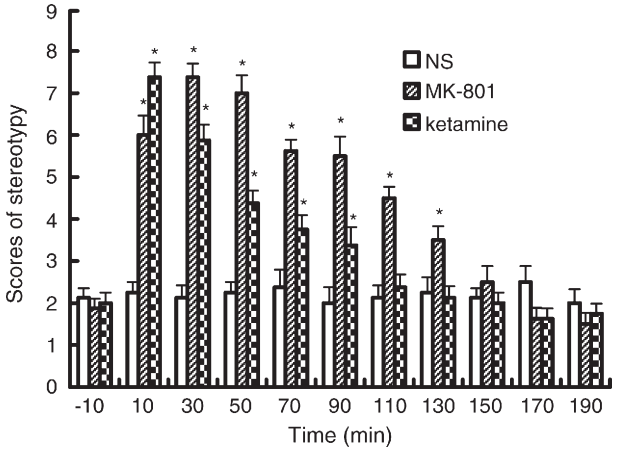

Fig. 5. Effect of MK-801 (0.6 mg/kg i.p.), ketamine (50 mg/kg i.p.) or saline challenge on stereotyped behavior of mice treated with MK-801 at 0.6 mg/kg or ketamine at 50 mg/kg or saline per day respectively for 7 consecutive days. Data are expressed as the percentage of basal levels and are given as mean±S.E.M. (n=9). *p<0.05 compared with the corresponding vehicle group. Between the vehicle group and the MK-801 group, $F(1,14)=193.870$, $p<0.001$, between the vehicle group and the ketamine group, $F(1,14)=27.172$, $p<0.001$ (two-way ANOVA for repeated measurements).

To our knowledge, this is the first report on the in vivo influence of systemically administered MK-801 and ketamine on the extracellular levels of ·OH in the PC/RS cortex of freely moving mice measured via intracerebral microdialysis. ·OH is generated during normal metabolic processes by incomplete reduction of oxygen to $\cdot O_2^-$ and H_2O_2 by the Fenton reaction in the presence of transitional metals (Coyle and Puttfarcken, 1993). In excess, ·OH can produce cellular damage by oxidizing proteins, lipids and nucleic acids. Regions, such as the brain, which have high rate of oxidative metabolic activity and high lipid content are at particular risk of oxidative damage (Mahadik and Mukherjee, 1996). One of the most significant findings in this study is the in vivo generation of ·OH in the PC/RS cortex following acute and chronic administration of NMDA receptor antagonists. Pronounced increases of ·OH levels suggest that NMDA receptor antagonists induce ·OH formation in the PC/RS cortex of mice. Increased production of ·OH in presence of NMDA receptor antagonists is interesting and raises the question as to how this happens. While the precise mechanism of ·OH generation by the action of NMDA receptor antagonists is obscure, increased production of ·OH in the PC/RS cortex may be elucidated at least by three biochemical mechanisms. One mechanism is the increased production of activated oxygen species, such as superoxide and H_2O_2. Another mechanism is linked to the decrease in activity of enzymes to scavenge the activated oxygen, such as superoxide dismutase (SOD) and catalase. The third mechanism may be the reduction of reduced glutathione (GSH) and its recycling enzymes, glutathione peroxidase and glutathione reductase (Ohkuwa et al., 1995).

A number of studies have demonstrated that systemic administration of NMDA receptor antagonists like MK-801 and ketamine can induce structural damage, reversible or irreversible neuronal alterations in the PC/RS cortex (Brosnan-Watters et al., 1999; Li et al., 2002; Olney et al., 1991, 1999). Furthermore, Rajdev et al. (1998) have previously shown that neurotoxicity and expression of haem oxygenase-1 (HO), a heat shock protein induced by oxidative stress, can be induced by PCP in the PC/RS cortex of rat. Pretreatment with 1,3-dimethylthiourea, an antioxidant, significantly reduced this neurotoxicity and expression of HO. Their results suggested that the neurotoxicity of NMDA antagonists in the PC/RS cortex was due in part to the oxidative stress. ·OH is a production of oxidative stress. Consequently, in vivo generation of ·OH in the PC/RS cortex following the acute and chronic administration of NMDA receptor antagonists at least partly contribute to their neurotoxicity. On the other hand, the neuropathological damages of NMDA receptor antagonists might be partly mediated by a complex polysynaptic mechanism. NMDA receptor antagonists can interfere with GABAergic inhibition and thus produce disinhibition (Olney and Farber, 1995; Farber et al., 2002) and ultimately leading to the release of acetylcholine and glutamate. It has been suggested that excessive release of glutamate after systemic administration of NMDA receptor antagonists might contribute to neuropathological changes in rat PC/RS cortex (Farber et al., 2002; Moghaddam and Adams, 1998). Furthermore, recent in vivo experiments have clearly demonstrated that glutamate discharge

promotes the extracellular release of ·OH (Laplanche et al., 2000; Yang et al., 1995). As a result, the release of glutamate induced by NMDA receptor antagonists might be another important reason that contributes to the increase of ·OH in the PC/RS cortex.

It has been demonstrated that a profound increase in glucose utilization in various brain structures associated with the limbic system after treatment with NMDA receptor antagonists such as PCP and dizocilpine (Kurumaji et al., 1989; McCulloch and Iversen, 1991). The increased glucose utilization induced by NMDA receptor antagonists closely mirrors the pattern of neurotoxicity (review by Ellison, 1995) and the increased ·OH levels in the PC/RS cortex, which suggests that prolonged hyperactivity in these regions may contribute to the neuropathological changes and subsequently induce the production of ·OH in this region. On the other hand, it has been reported that NMDA receptor antagonists produce a marked expression of c-fos or heat shock protein in the corticolimbic regions, including the PC/RS cortex in the animal brain (Nakki et al., 1996; Nishizawa et al., 2000; Sharp et al., 1991). Consequently, it is likely that the marked expression of c-fos or heat shock protein in the PC/RS cortex produced by NMDA receptor antagonists is also associated with the ·OH changes in this region. However, it is not clear whether the increased ·OH occurs in the same cells that manifest pathomorphological changes, or in some other neural elements in this region, it seems likely that these phenomena are mechanistically interrelated. A further systematic study was necessary to elucidate their relationship.

NMDA antagonists produce psychosis and aggregate the symptoms of schizophrenic patients (Tsai and Coyle, 2002) and have been extensively used in animal models of schizophrenia. In light of the close relationship between NMDA antagonists and schizophrenia, and the fact that our study results show that MK-801 and ketamine increase ·OH production in the PC/RS cortex of mice, it is possible that the psychosis produced by these drugs and the worsening of schizophrenia in patients might be related to ·OH. On the other hand, some researchers report that increased free radicals are implicated in schizophrenia (Do et al., 2000; Mahadik and Mukherjee, 1996; Yao et al., 2001). Therefore, the production of ·OH by ketamine and MK-801 in the PC/RS cortex of mice could be relevant to the mechanisms of free radicals involved in patients with schizophrenia.

In rodents, one of the signs of neurotoxicity is stereotyped behavior. A good correlation between toxic effects of psychostimulant drugs and stereotyped behavior has been reported by Wallace et al. (1999). Although there are some studies on the effect of NMDA antagonist on stereotyped behavior of mice, to our knowledge, there is no report about repeated administration of MK-801 and ketamine on stereotypy behavior of mice and their comparative investigation in the literature. Consistent with several previous reports in mice or rats (Nishizawa et al., 2000; Andine et al., 1999), the present study found that MK-801 and ketamine acute administration induced a complex behavioral syndrome with hyperactivity and stereotyped behaviors consisting of head weaving, turning and repetitive exploration of the cage. All behavioral effects occurred a few minutes after administration. Although both MK-801 and ketamine produced stereotyped behavior in mice, its severity was different with ketamine being less pronounced. Compared with MK-801, the maximal increase of ketamine induced stereotyped behavior was earlier and the increase duration was shorter. In contrast to acute administration, the stereotyped behaviors induced by chronic MK-801 or ketamine treatment showed more sensitive. In order to know whether there was correlation between individual ·OH levels and stereotyped behavior, Pearson's correlation coefficients were calculated in each mouse after MK-801 or ketamine administration. However, our results suggested that there was not significant correlation in all mice (data were not shown). Our results showed that the release of ·OH did not happen simultaneously with the stereotyped behavior. The former lagged behind the latter, i.e., the release of ·OH was secondary to stereotyped behavior.

Altogether, these results provide evidence suggesting that NMDA receptor antagonists participate to the generation of ·OH in the PC/RS cortex of mice, and oxidative stress, derived from the formation of free radicals, may be involved in NMDA receptor antagonists-induced neurotoxicity in the PC/RS cortex of mice. An involvement of ·OH in the animal model of schizophrenia induced by NMDA receptor antagonists also indirectly supports the hypothesis that free radicals play an important role in schizophrenia. Further elucidation of their relationship will require systematic investigation.

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