International Journal of Neuropsychopharmacology (2007), **10**, 359–368. Copyright © 2006 CINP
doi:10.1017/S1461145706006869

ARTICLE

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The effects of repeated administrations of MK-801 on ERK and GSK-3β signalling pathways in the rat frontal cortex

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Abstract

Repeated administrations of NMDA receptor antagonists induce behavioural changes which resemble the symptoms of schizophrenia in animals. ERK and GSK-3β associated signalling pathways have been implicated in the pathogenesis of psychosis and in the action mechanisms of various psychotropic agents. Here, we observed the phosphorylations of ERK and GSK-3β and related molecules in the rat frontal cortex after repeated intraperitoneal injections of MK-801, over periods of 1, 5, and 10 d. Repeated treatment with 0.5, 1, and 2 mg/kg MK-801 increased the phosphorylation levels of the MEK-ERK-p90RSK and Akt-GSK-3β pathways and concomitantly and significantly increased CREB phosphorylation in the rat frontal cortex. However, single MK-801 treatment did not induce these significant changes. In addition, the immunoreactivities of HSP72, Bax, and PARP were not altered, which suggests that neuronal damage may not occur in the rat frontal cortex in response to chronic MK-801 treatment. These findings suggest that chronic exposure to MK-801 may induce pro-survival and anti-apoptotic activity without significant neuronal damage in the rat frontal cortex. Moreover, this adaptive change might be associated with the psychotomimetic action of MK-801.

Received 29 July 2005; Reviewed 1 September 2005; Revised 23 February 2006; Accepted 6 April 2006; First published online 19 June 2006

Key words: Akt, CREB, MAPK, NMDA receptor antagonist, psychotomimetics.

Introduction

MK-801 is a non-competitive, selective NMDA receptor antagonist, and has been demonstrated to induce psychotomimetic behaviour in rodents. The single administration of an NMDA receptor antagonist was found to result in acute psychotomimetic behavioural effects, including locomotor activity and stereotypy (Andine et al., 1995). On the other hand, prolonged treatment of rats with NMDA receptor antagonists were found to induce behavioural effects, which are consistent with the cognitive deficits and negative symptoms of schizophrenia, and sensitized psychotomimetic behaviour. Therefore, the chronic exposure of rats to NMDA receptor antagonist may provide a more appropriate animal model than acute exposure since it results in adaptive responses that model in certain aspects of the schizophrenic state in humans (Jentsch and Roth, 1999).

NMDA receptor antagonists induce functional alterations in the brain, and the NMDA receptor hypofunctional state induces aberrations in the GABAergic, serotonergic, adrenergic, and dopaminergic neurotransmission systems (Farber, 2003), and chronic exposure to phencyclidine (PCP) has been reported to generate compensatory hyperfunctional NMDA receptors (Yu et al., 2002). Blood flow, dopamine neurotransmission, and glucose utilization, which together represent brain functional activity, can

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also be influenced by NMDA receptor antagonist treatment (Jentsch and Roth, 1999). On the other hand, NMDA receptor antagonists also affect the fates of neuronal cells. Although many studies have indicated that neuronal damage occurs in response to NMDA receptor antagonists (Carter et al., 2004; Sharp et al., 1991; Tomitaka et al., 2000), NMDA receptor antagonists can also protect neurons from various stimuli including excitotoxicity (Molinuevo et al., 2005; Thomas and Kuhn, 2005). Moreover, these functional and structural changes induced by NMDA receptor antagonists can result from the actions of intracellular signalling pathways. However, the action mechanisms of NMDA receptor antagonists on intracellular signalling pathways in vivo, altering the activity of neural system has yet to be clarified.

Although the intracellular mechanisms underlying the psychotomimetic effects of NMDA antagonists remain poorly understood, the Ras-MAPK kinase (MEK)-MAPK and the PI3 kinase-Akt-GSK-3β signalling pathways have been suggested to be the primary signalling pathways exploited by NMDA receptors (Grewal et al., 1999; Zhu et al., 2005). It has been reported that ERK1/2 and Akt can be activated by NMDA receptor activation, and that MK-801 treatment reduces the phosphorylation levels of ERK1/2 and Akt in cortical neuronal cultures (Bading and Greenberg, 1991; Chandler et al., 2001; Sutton and Chandler, 2002).

The Ras-MAPK kinase (MEK)-MAPK and the PI3 kinase-Akt-GSK-3β signalling pathways have been implicated in both the pathogenesis and in the therapeutic mechanisms associated with the treatment of psychosis. Reduced protein levels and GSK-3β activities in the frontal cortex (Beasley et al., 2001; Kozlovsky et al., 2001), and increased ERK2 protein and mRNA levels in the thalamus (Kyosseva, 2004) have been revealed by post-mortem studies of schizophrenia. The pathway involving GSK-3β was also suggested to be a common signalling pathway on which various psychotomimetic agents act (Svenningsson et al., 2003). Moreover, antipsychotics such as clozapine and olanzapine, and electroconvulsive shock treatment (ECS) have been shown to increase the phosphorylation of GSK-3β and ERK in both cell lines and in rat brains (Browning et al., 2005; Kang et al., 2004; Lu et al., 2004; Roh et al., 2003).

Recently, our group reported that a single treatment with MK-801 results in acute dose-dependent phosphorylation changes in the Akt-GSK-3β and c-Raf-MEK-ERK signalling pathways in the rat frontal cortex, and associated these phosphorylation changes with the behavioural changes induced by MK-801 (Ahn et al., 2005a,b). Although the evaluation of possible changes in these pathways after repeated applications of MK-801, will provide necessary information regarding the intracellular mechanisms underlying the psychotomimetic effects exerted by MK-801, it cannot be expanded from the results of acute experiments alone (Jentsch and Roth, 1999).

In this study, we observed changes in GSK-3β and ERK1/2 related molecules in the rat frontal cortex after repeated intraperitoneal (i.p.) injections of 0.5, 1, and 2 mg/kg MK-801. Changes in these pathways can be associated with alterations in the fates of neuronal cells and with altered brain functional activities induced by repeated MK-801 treatment. Here, we found that chronic MK-801 treatment up-regulates the Akt-GSK-3β and MEK-ERK-p90RSK pathways and concurrently activates CREB in the rat frontal cortex. These findings suggest that the pro-survival activity of signalling pathways is enhanced by chronic exposure to MK-801.

Materials and Methods

Animals and drug treatment

Male Sprague–Dawley rats (150–200 g) were separated into groups (described below), and maintained on a 12-h light–dark cycle with food and water available ad libitum. All animal treatments were consistent with the protocols described in the NIH Guide for the Care and Use of Laboratory Animals. MK-801 (Tocris, St. Louis, MO, USA), dissolved in normal saline was administered by i.p. injection to treated animals, whereas saline-treated control (Vehicle) animals received injections containing equivalent volumes of normal saline. Sham (Normal control) animals were treated in precisely the same way but were not injected intraperitoneally.

MK-801 dosages were decided upon based on our previous acute experimental findings (Ahn et al., 2005a,b). In our previous acute experiments, MK-801 dosages of 0.5, 1, and 2 mg/kg induce different levels of psychotomimetic behavioural change. In the frontal cortex, MK-801 at 0.5 mg/kg increased Akt-GSK-3β phosphorylation, but reduced MEK1/2-ERK1/2 phosphorylation at 1 h after a single MK-801 injection. MK-801 at 1 mg/kg also induced the same pattern of phosphorylation changes, with maximal Akt-GSK-3β phosphorylation. However, 2 mg/kg MK-801 elevated the phosphorylation levels of Akt-GSK-3β and MEK1/2-ERK1/2. Therefore, we attempted to determine the effects of 0.5, 1, and 2 mg/kg MK-801 on GSK-3β- and ERK-related molecules in the rat frontal cortex after repeated MK-801 treatment.

Rats were given a single injection (of MK-801 or normal saline) or repeated injections (of MK-801 or normal saline) for 5 or 10 consecutive days (one injection per day at the same time of the day) [abbreviation: 1× (single injection), 5×, and 10× (repeated injections)]. Animals were sacrificed by decapitation 24 h after final injections to examine chronic changes and rat frontal cortices were excised and immediately (<2 min) frozen in liquid nitrogen to exclude the possible confounding effect of GSK-3 phosphorylation changes (Li et al., 2005) until required for analysis.

Western blot analysis

Whole extracts of frontal cortex were used for Western blot analysis. Frontal cortices were immediately homogenized in RIPA(+) (0.1% SDS, 1.0% Triton, 1.0% deoxycholate, 0.1% NaN3 in PBS) containing 5 mm DTT, 1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA), and 1 mm PMSF (Sigma-Aldrich) for 15 min at 4 °C. Protein concentrations were quantified using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Homogenates were then centrifuged, and supernatants were boiled in Laemmli's sample buffer. Equal quantities of proteins were separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE), and the resulting gels were then either stained with Coomassie Blue, or transferred to nitrocellulose membranes (Bio-Rad Laboratories). For Western blotting, membranes were blocked with 5% skim milk in TBS-T (0.1% Tween-20 in TBS) for 1 h at room temperature, on a shaker and then incubated with anti-actin (Sigma-Aldrich), anti-phospho-GSK-3 (Tyr216 of GSK-3β/Tyr297 of GSK-3α) (Upstate Biotechnology, Lake Placid, NY, USA), anti-HSP72 (C92, StressGen, Victoria, BC, Canada), anti-GSK-3, anti-CREB, anti-ERK1/2, anti-MEK1/2, anti-c-Raf, anti-p90RSK, anti-Bax (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-PARPP, anti-phospho-GSK-3β (Ser9), anti-phospho-GSK-3α (Ser21), anti-phospho-Akt (Ser473), anti-phospho-Akt (Thr308), anti-phospho-CREB (Ser133), anti-phospho-ERK1/2 (Thr202/Tyr204), anti-phospho-MEK1/2 (Ser217/Ser221), anti-phospho-c-Raf (Ser259), anti-phospho-c-Raf (Ser338), and anti-phospho-p90RSK (Ser380) (Cell Signaling Technology, Beverly, MA, USA) specific antibodies, at 1:1000–3000 dilutions at 4 °C overnight. The membranes were then incubated with horseradish peroxidase-conjugated anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and signals were detected using an ECL system (Pierce, Rockford, IL, USA). Film exposures were adjusted as determined by preliminary experiments.

Immunohistochemistry

Different animals were used for immunohistohemistry from the animals used for Western blot analysis. However, they were treated in the same way except the methods of analysis. Rats received injections (1 mg/kg MK-801 i.p.), and were then transcardially perfused with 200 ml saline solution, followed by 500 ml of 4% paraformaldehyde (Sigma-Aldrich) in 0.01 M PBS (pH 7.4). Brains were then immediately removed and cryoprotected in 20% sucrose solution at 4 °C for 24 h. Cryoprotected brains were sectioned at 20 μm on a freezing microtome (Leitz, Wetzlar, Germany). In order to perform immunohistochemistry, we used the FR1 and FR2 areas of the frontal cortex (Paxinos and Watson, 1998), and sections were incubated with phospho-Thr202/Tyr204-ERK1/2 and phospho-Ser9GSK-3β (Cell Signaling Technology) at a 1:10000 dilution for 7 d at 4 °C and then incubated with FITC-488 conjugated secondary antibody (1:200; Molecular Probes, Eugene, Oregon, USA) for 2 h at room temperature. The sections were then fluorescence-labelled, and were also counterstained with DAPI (0.4 μl/ml; Sigma-Aldrich), and then examined by fluorescence microscopy.

Statistical analysis

Three animals were assigned to each treatment group [Sham, vehicle, 0.5, 1, and 2 mg/kg MK-801, single/repeated (for 1, 5 and 10 d)]. Results are expressed as relative optical densities, and are presented as mean ± S.E. The mean relative optical densities of each immunoreactivity of molecules were compared with the vehicle control value of each group using analysis of variance (ANOVA) (Figures 1, 2, 4, and 5). In order to determine dose dependency effects, data were analysed using two-way ANOVA followed by the Student–Newman–Keuls post-hoc test with respect to doses of MK-801 and treatment length (Figure 4). p values of <0.05 were considered statistically significant. All tests were performed using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Repeated treatment with 1 mg/kg MK-801 for 5 d was found to increase the immunoreactivities of p-Ser473-Akt and p-Ser9-GSK-3β in the rat frontal cortex at 24 h after the final injection. After 10 d of repeated injections, the phosphorylations of these molecules were also increased in the rat frontal cortex again at 24 h after the final injection. However, p-Ser473-Akt and p-Ser9-GSK-3β were unchanged 24 h after a single

injection of 1 mg/kg MK-801. The p-Ser473-Akt immunoreactivity levels in the 5-d and 10-d treatment groups were 248% and 254% of those of the corresponding vehicle controls ($F=50.14$, d.f. $=1$, $p<0.01$ and $F=181.05$, d.f. $=1$, $p<0.01$). The p-Ser9-GSK-3β immunoreactivity levels of the 5-d and 10-d treatment groups were 305% and 277% of the vehicle control values ($F=197.66$, d.f. $=1$, $p<0.01$ and $F=107.03$, d.f. $=1$, $p<0.01$). The immunoreactivities of total Akt, GSK-3β, and GSK-3α were unchanged after repeated injections with 1 mg/kg MK-801 and the immunoreactivities of p-Thr308-Akt, p-Ser21-GSK-3α, p-Tyr216-GSK-3β, and p-Tyr297-GSK-3α were not affected at 24 h after the repeated injections of 1 mg/kg MK-801 (Figure 1).

The immunoreactivities of p-Ser217/Ser221-MEK1/2, p-Thr202/Tyr204-ERK1/2, and p-Ser380-p90RSK were increased in frontal cortices in the 5-d and 10-d treatment groups (1 mg/kg MK-801), but were unchanged in the 1-d treatment group (1 mg/kg MK-801). p-Ser217/Ser221-MEK immunoreactivity levels in the 5-d and 10-d treatment groups were 244% and 284% of the vehicle control levels ($F=17.346$, d.f. $=1$, $p=0.01$ and $F=105.88$, d.f. $=1$, $p<0.01$). Immunoreactivity levels of p-Thr202/Tyr204-ERK1/2 in the 5-d and 10-d treatment groups were 259% and 232% of the vehicle control levels, respectively ($F=130.74$, d.f. $=1$, $p<0.01$ and $F=30.70$, d.f. $=1$, $p<0.01$). p-Ser380-p90RSK immunoreactivity levels in the 5-d and 10-d treatment groups were 224% and 264% of that of the vehicle controls ($F=53.41$, d.f. $=1$, $p<0.01$ and $F=92.42$, d.f. $=1$, $p<0.01$). The immunoreactivities of total MEK, ERK, and p90RSK were unchanged after repeated 1 mg/kg MK-801 treatment in any group, and the phosphorylations of c-Raf on Ser259 and Ser338 remained unchanged in the 5-d and 10-d treatment groups (Figure 2).

Phospho-Ser133-CREB immunoreactivity was also elevated in the frontal cortex in the 5-d and 10-d treatment groups (1 mg/kg MK-801), but was unchanged in the 1-d treatment group (1 mg/kg MK-801). Immunoreactivity levels of p-Ser133-CREB in the 5-d and 10-d treatment groups were 267% and 243% of the vehicle control values, respectively ($F=69.21$, d.f. $=1$, $p<0.01$ and $F=128.66$, d.f. $=1$, $p<0.01$). However, total CREB immunoreactivity was unchanged after repeated injection with 1 mg/kg of MK-801 (Figure 2).

In order to confirm the immunoblot findings, immunohistochemical analysis was conducted on rat frontal cortical tissues. Increased p-Ser9-GSK-3β and p-Thr202/Tyr204-ERK1/2 immunoreactivities were observed in the 10-d treatment group (1 mg/kg MK-801). Based on cell morphology observations, neurons in the frontal cortex were observed to contain both p-Ser9-GSK-3β and p-Thr202/Tyr204-ERK1/2. The number of neuronal cells densely stained for p-Ser9-GSK-3β and p-Thr202/Tyr204-ERK1/2 were higher in the 10-d treatment group than in the vehicle control group (Figure 3).

As mentioned previously, 0.5, 1, and 2 mg/kg MK-801 induced different pattern of acute changes in

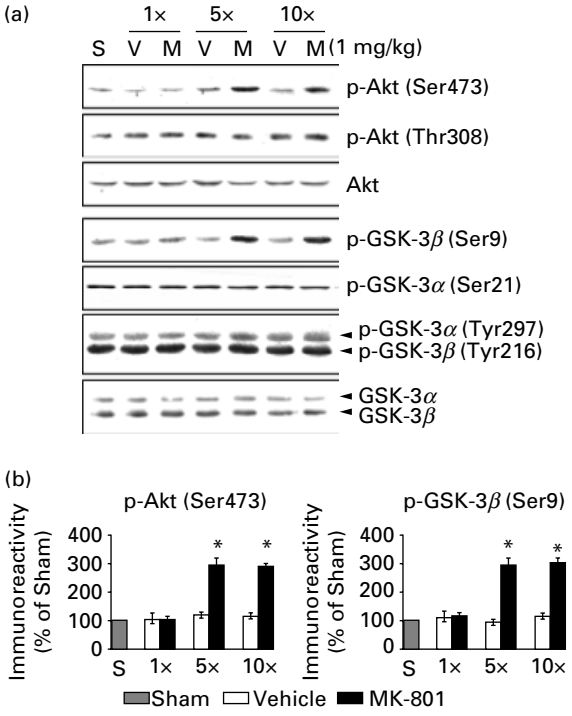

Figure 1. Increased immunoreactivity of p-Ser473-Akt and p-Ser9-GSK-3β induced by repeated treatments with 1 mg/kg MK-801 in the rat frontal cortex. (a) Immunoblots of rat frontal cortex after repeated treatments with MK-801 (1 mg/kg) for 1, 5, or 10 d. The immunoreactivities of p-Ser473-Akt and p-Ser9-GSK-3β were increased vs. the vehicle controls in the 5-d and 10-d treatment groups. The immunoreactivities of Akt, p-Thr308-Akt, GSK-3α, GSK-3β, p-Tyr216-GSK-3β, and p-Tyr297-GSK-3α were similar to those of the vehicle controls after 1-d, 5-d, and 10-d MK-801 treatment. (b) Comparison of immunoblot and densitometry results. Mean optical densities (ODs) of p-Ser473-Akt and p-Ser9-GSK-3β were significantly elevated vs. vehicle control in the 5-d and 10-d MK-801 treatment groups. Data are expressed as relative ODs, and represent means $\pm$ S.E. Relative ODs are quoted as percentages vs. the ODs of the sham (normal untreated controls). S and V indicate the normal and vehicle controls respectively. The asterisks (*) denote significant differences between the OD of the MK-801 treatment group and the OD of the vehicle controls ($p<0.05$).

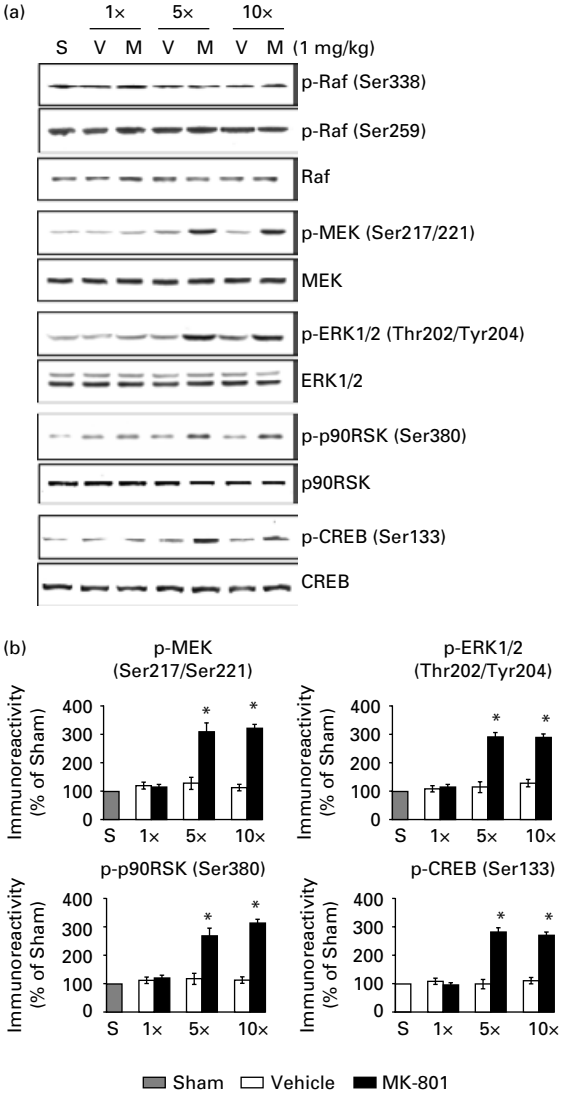

Figure 2. Increased immunoreactivities of p-Ser217/Ser221-MEK1/2, p-Thr202/Tyr204-ERK1/2, p-Ser380-p90RSK, and p-Ser133-CREB induced by repeated treatment with 1 mg/kg MK-801 in the rat frontal cortex. (a) Immunoblots of rat frontal cortex after repeated MK-801 (1 mg/kg) treatment for 1, 5, or 10 d. Immunoreactivities of p-Ser217/Ser221-MEK1/2, p-Thr202/Tyr204-ERK1/2, p-Ser380-p90RSK, and p-Ser133-CREB were increased vs. the vehicle controls in the 5-d and 10-d treatment groups. The immunoreactivities of MEK1/2, ERK1/2, p90RSK and CREB were similar to those of the vehicle controls in the 1-d, 5-d, and 10-d groups. (b) Quantification of immunoblot data by densitometric analysis. The mean optical densities of p-Ser217/Ser221-MEK1/2, p-Thr202/Tyr204-ERK1/2, p-Ser380-p90RSK, and p-Ser133-CREB were significantly increased vs. those of the vehicle controls in the 5-d and 10-d treatment groups. Quantification of immunoblot data by densitometric analysis performed as described in the Figure 1 legend.

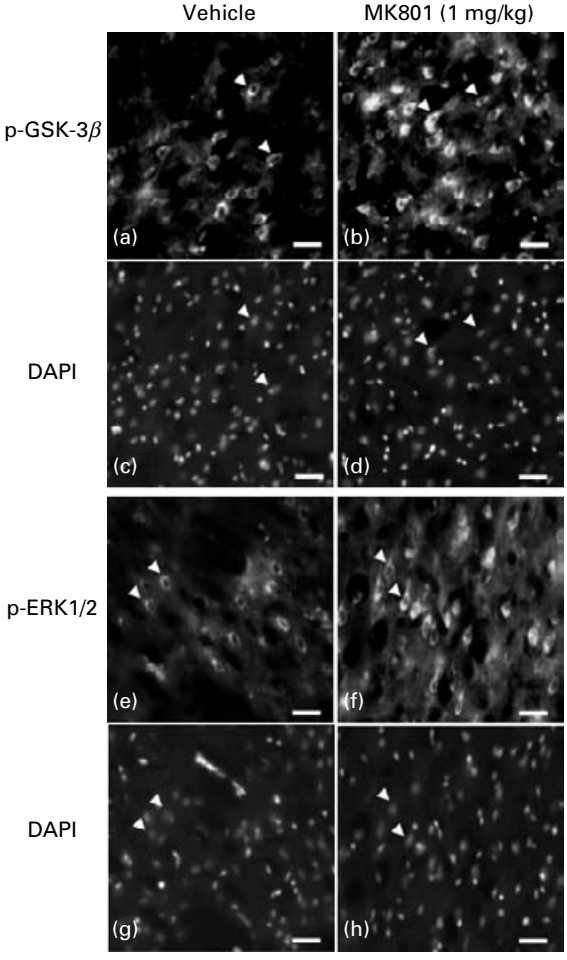

Figure 3. Increased immunostaining of p-Ser9-GSK-3β and p-Thr202/Tyr204-ERK in rat prefrontal cortices after 10-d treatment with MK-801 (1 mg/kg). Representative fluorescence microscopic images taken from sections stained for p-Ser9-GSK-3β [(a) and (b)] or p-Thr202/Tyr204-ERK1/2 [(e) and (f)], and counterstained with DAPI to label nuclei [(c), (d), (g) and (h)]. All captured images were obtained 24 h after repeated daily treatment with MK-801 (1 mg/kg) [(b), (d), (f) and (h)] or saline [(a), (c), (e) and (g)] for 10 d. Phospho-Ser9-GSK-3β and phospho-Thr202/Tyr204-ERK1/2 stainings coincided with DAPI nuclear staining (arrowheads). Phospho-Ser9-GSK-3β and phospho-Thr202/Tyr204-ERK1/2 stainings were increased by increasing MK-801 (arrowheads). Magnification bar = 50 μm.

the phosphorylations of ERK- and GSK-3β-related molecules at 1 h after intraperitoneal injection of MK-801. In order to confirm this dose-dependency in the chronic experiments, we also examined the immunoreactivities of p-Ser473-Akt, p-Ser9-GSK-3β, p-Ser217/Ser221-MEK1/2, p-Thr202/Tyr204-ERK1/2, p-Ser380-p90RSK, and p-Ser133-CREB in the rat frontal cortex after repeated injections with 0.5 and

2 mg/kg MK-801. Immunoreactivities of these phosphoproteins were all determined to have increased significantly at 24 h after 5 d and 10 d repeated treatments with 0.5 and 2 mg/kg MK-801 (all $p < 0.05$). The findings with 0.5 and 2 mg/kg MK-801 showed same pattern with those with 1 mg/kg MK-801. All optical densities of these molecules' immunoreactivities with 0.5, 1, and 2 mg/kg MK-801 showed significant increase compared to the vehicle controls value of each treatment length group, whereas there were no significant differences among immunoreactivities of each molecule with 0.5, 1, and 2 mg/kg MK-801 within each treatment length group. This finding demonstrates that there was no dose-dependency with repeated treatments with MK-801 (0.5, 1, and 2 mg/kg) (Figure 4).

In addition, immunoreactivities of HSP72, PARP, and Bax were evaluated to investigate whether neuronal damage occurred in response to repeated treatments of MK-801. There were no significant differences between the vehicle group and the MK-801-treated group with regard to the HSP72, PARP, and Bax immunoreactivity in the rat frontal cortex, 24 h after 1, 5, and 10 daily repeated injections with 1 and 2 mg/kg MK-801 (Figure 5).

Discussion

The most important finding of the present study is that repeated treatments with 1 mg/kg MK-801 resulted in the up-regulation of phosphorylation levels of the Akt-GSK-3β and MEK-ERK-p90RSK pathways in the rat frontal cortex. However, single MK-801 treatment did not induce significant changes in these pathways. CREB phosphorylation was also increased in the 5× and 10× groups, and the same pattern of changes in these molecules were found in the corresponding 0.5 and 2 mg/kg MK-801 groups. We also found that the level of HSP-72 protein, a known marker of neuronal death (Sharp et al., 1991), was not increased as a result of these treatments.

It is interesting that the above alterations in the Akt-GSK-3β and MEK-ERK-p90RSK pathways were not observed after a single administration. Kyosseva et al. (2001) reported that PCP increased MEK and ERK1/2 phosphorylations in the cerebellum of rats after 10-d and 20-d treatment, whereas 3 d of PCP treatment did not result in any significant increases in their phosphorylations. In contrast to these chronic changes, we previously observed acute changes, at 1 h after a single injection of MK-801, on the phosphorylation levels of Akt-GSK-3β and MEK-ERK pathways, and the responses of these pathways were

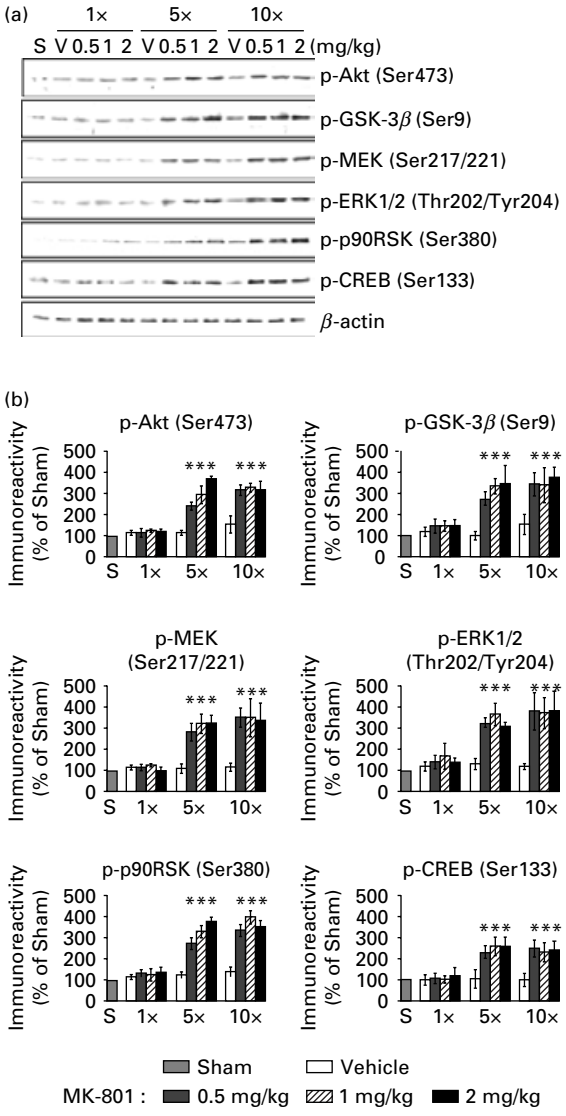

Figure 4. Increased immunoreactivities of p-Ser473-Akt, p-Ser9-GSK-3β, p-Ser217/Ser221-MEK1/2, p-Thr202/Tyr204-ERK1/2, p-Ser380-p90RSK, and p-Ser133-CREB in the rat frontal cortex induced by repeated daily treatment with 0.5, 1, or 2 mg/kg MK-801. (a) Immunoblots of rat frontal cortex after repeated exposure to MK-801 at the indicated doses. The immunoreactivities of p-Ser473-Akt, p-Ser9-GSK-3β, p-Ser217/Ser221-MEK1/2, p-Thr202/Tyr204-ERK1/2, p-Ser380-p90RSK, and p-Ser133-CREB were increased in the rat frontal cortex 24 h after 5-d and 10-d daily treatment with 0.5, 1, and 2 mg/kg MK-801. (b) Quantification of immunoblot data by densitometry. The mean optical densities of p-Ser473-Akt, p-Ser9-GSK-3β, p-Ser217/Ser221-MEK1/2, p-Thr202/Tyr204-ERK1/2, p-Ser380-p90RSK, and p-Ser133-CREB were significantly higher than those of the 2vehicle control after 5-d and 10-d MK-801 treatment. Quantification of immunoblot data by densitometry performed as described in the Figure 1 legend.

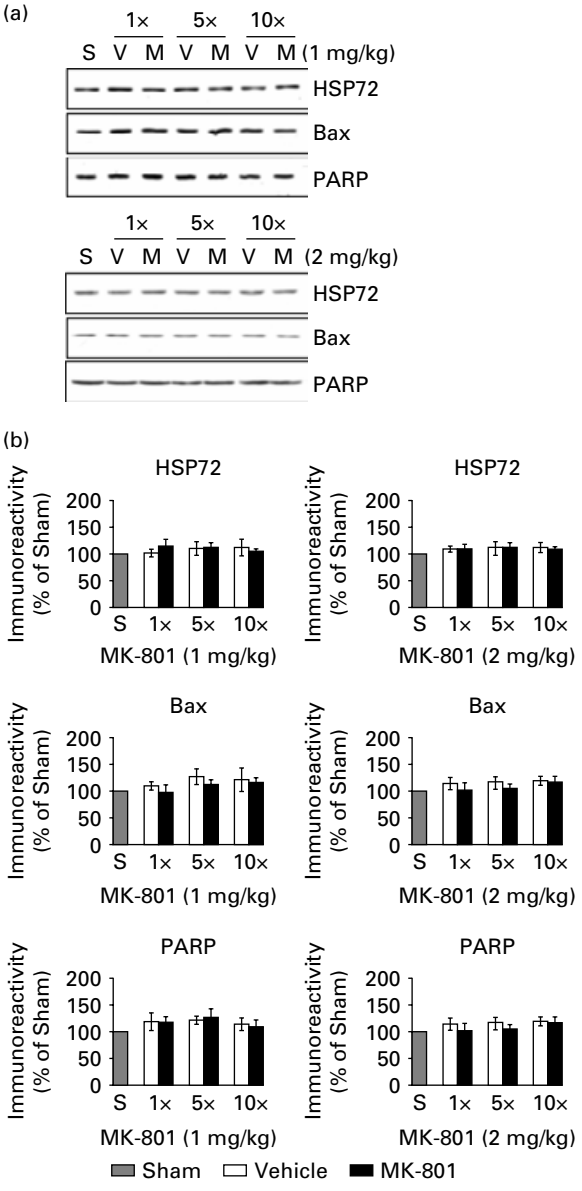

Figure 5. No significant changes occurred in the immunoreactivities of HSP72, Bax, or PARP in rat frontal cortices after repeated MK-801 (1 and 2 mg/kg) treatment. (a) Immunoblots of rat frontal cortex after repeated treatments with MK-801 (1 and 2 mg/kg) for 1, 5, and 10 d. Immunoreactivities of HSP72, Bax, and PARP were similar to those of the vehicle controls in the 1-d, 5-d, and 10-d groups. (b) Quantification of immunoblot data by densitometry. The mean optical densities of HSP72, Bax, and PARP were similar to those of the vehicle controls in the 5-d and 10-d groups. Quantification of immunoblot data by densitometry performed as described in the Figure 1 legend.

different from each other (Ahn et al., 2005a,b). These findings suggest that alterations in intracellular signalling pathways induced by NMDA receptor antagonists are dependent on the duration of exposure and that this reflects an adaptive response to a chronic NMDA receptor hypofunctional state.

Our finding that MK801, an NMDA receptor antagonist, increases the phosphorylations of Akt and ERK seems to contradict to reports that MK-801 reduces NMDA receptor-mediated ERK and Akt phosphorylations in primary cortical cell cultures (Chandler et al., 2001; Sutton and Chandler, 2002; Zhu et al., 2005). This apparent discrepancy may be due to compensatory changes induced by NMDA receptor antagonist treatment in the brain, as MK-801 can induce alterations in GABAergic, serotonergic, and adrenergic neurotransmission systems (Farber, 2003; Olney and Farber, 1995). Moreover, chronic treatments with PCP can induce the synthesis of new synaptic hyperfunctional NMDA receptors (Yu et al., 2002). Therefore, the increased phosphorylations of Akt and ERK may be related to a mechanism independent of NMDA receptor antagonism by MK-801.

The PI3K-Akt and MEK-ERK pathways are the principal pathways that contribute to neuronal survival (Hetman and Gozdz, 2004; Zhu et al., 2005; Zimmermann and Moelling, 1999). CREB exerts its pro-survival effect by mediating Akt and ERK signalling (Walton and Dragunow, 2000). The activation of p90RSK is necessary for CREB to mediate ERK signalling (Frodin and Gammeltoft, 1999; Xing et al., 1996), and we found that Ser380-p90RSK phosphorylation was increased after repeated MK-801 treatment with a similar pattern with ERK1/2 and CREB phosphorylation. Of the many phosphorylation sites on p90RSK, Ser380 phosphorylation is known to be important for activating the N-terminal kinase domain to phosphorylate substrates of p90RSK, including CREB (Dalby et al., 1998; Frodin and Gammeltoft, 1999). Therefore, it appears that Ser133-CREB phosphorylation is mediated by MEK-ERK-p90RSK signalling in response to repeated MK-801 treatment. In addition, the apoptosis-inducing property of GSK-3β is inhibited by its phosphorylation at Ser9 by Akt (Cross et al., 1995). Akt-GSK-3β signalling can also facilitate CREB activation, but this activation is mediated by phosphorylation at Ser129, not at Ser133 (Grimes and Jope, 2001). Taken together, chronic MK-801 treatment activates the Akt-GSK-3β and the MEK-ERK-p90RSK-CREB pathways, which suggests that pro-survival and anti-apoptotic activities of these signalling pathways are enhanced in response to chronic exposure to MK-801.

The immunoreactivities of HSP72, Bax, and PARP remained unchanged in the rat frontal cortex, even after repeated injections of 2 mg/kg MK-801. It is

known that HSP72 can be used to detect neuronal injury (Carter et al., 2004; Sharp et al., 1991; Tomitaka et al., 2000), and that HSP72 immunoreactivity (determined by Western blotting) may reflect neuronal damage in brain tissue caused by NMDA receptor antagonist treatment (Tomitaka et al., 2000). Bax and PARP are constituents of the intrinsic caspase-mediated cell-death pathway (Ferrer and Planas, 2003). Moreover, although it cannot completely preclude the possibility of neuronal damage, it appears that neuronal damage did not occur in the rat frontal cortex in the present study, which may be related to the activation of the pro-survival and anti-apoptotic activities of signalling pathways. However, NMDA receptor antagonists, such as MK-801, PCP, and ketamine, have been reported to induce neuronal damage in the corticolimbic regions of the rat brain (Carter et al., 2004; Olney and Farber, 1995; Tomitaka et al., 2000). In the present study, the absence of observed changes in the immunoreactivities of HSP, Bax, and PARP can be attributable to several factors. First, male rats have been reported to be more resistant to neuronal damage by MK-801 than female rats (Fix et al., 1995), and the present experiments were conducted on male rats. Second, we analysed the rat frontal cortex. However, the regions reported to show neuronal damage induced by NMDA receptor antagonists are; the posterior cingulate cortex, retrosplenial cortex, and the limbic cortex (Carter et al., 2004; Corso et al., 1997; Sharp et al., 1991; Tomitaka et al., 2000). Thus, gender and brain regional differences could account for the observed discrepancy.

Recently, we reported that the behavioural changes induced by MK-801 could be associated with the phosphorylation levels of ERK and Akt pathways in the rat frontal cortex (Ahn et al., 2005a,b). MEK inhibitor (SL327), which blocks the ERK pathway in the brain, has been reported to increase locomotion (Einat et al., 2003). Moreover, repeated MK-801 administrations have been reported to induce behavioural sensitization; a phenomenon characterized by the progressive enhancement of the drug's ability to stimulate locomotion and stereotypy, which are considered to be psychotomimetic behavioural changes (Carey et al., 1995; Wedzony et al., 1993; Yang et al., 2002). Although we did not measure behavioural changes in this study, we did observe that the locomotor activity became more prominent and rapid on increasing the number of MK-801 injections. Carey et al. (1995) demonstrated that repetitive MK-801 treatments changed behavioural baselines, rather than simply enhancing the behavioural responses elicited by MK-801 treatment. This behavioural baseline shift was considered to be adaptive change induced by repetitive MK-801 treatment. Therefore, the activations of the ERK and Akt signalling pathways may be the adaptive response in intracellular signal transduction pathways related to the behavioural changes induced by chronic exposure to MK-801.

In addition, NMDA receptor antagonists, such as, MK-801, ketamine, and riluzole, have been suggested to have antidepressant effects (Berman et al., 2000; Papp and Moryl, 1994; Zarate et al., 2003), and the ERK and GSK-3β signalling pathways have been reported to be intracellular targets of antidepressant action. Lithium is a well known GSK-3β inhibitor, and other GSK-3β inhibitors have been shown to be able to elicit rapid antidepressant-like effects (Gould et al., 2004; Kaidanovich-Beilin et al., 2004). Fluoxetine was reported to increase ERK1/2 expression in the frontal cortex (Gould et al., 2004), but another study indicated that fluoxetine inhibits ERK1/2 phosphorylation in the hippocampus and frontal cortex (Fumagalli et al., 2005). Moreover, imipramine reduced the pathophysiologically induced dephosphorylation of GSK-3β (Roh et al., 2005), or increased ERK1 phosphorylation in the rat frontal cortex (Fumagalli et al., 2005). These results suggest the drug-specific effects of ERK and GSK-3β. The increased phosphorylations of ERK and GSK-3β observed in the present study may also be related to the antidepressant-like effect of MK-801, and further studies are necessary to clarify this possible relationship.

In summary, repeated treatment with MK-801 increased the activities of the MEK-ERK-p90RSK-CREB and Akt-GSK-3β pathways in the rat frontal cortex, without inducing significant neuronal damage. This observed prolonged up-regulation of pro-survival and anti-apoptotic activities indicates the induction of adaptive changes in intracellular signalling pathways to the chronic NMDA receptor hypofunctional state, which could be related to the schizophrenia-like effects of chronic MK-801 treatment. Our findings can facilitate understanding with regard to the action mechanism of NMDA receptor antagonists as psychotomimetic agents.

Acknowledgements

This research was supported by the Brain Research Center of the 21st Century Frontier Research Programme funded by the Ministry of Science and Technology (grant no. M103KV010007-05K2201-00730).

Statement of Interest

None.

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