ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol Exp 2006, 66: 215–226

Influence of dizocilpine (MK-801) on neurotoxic effect of dexamethasone: Behavioral and histological studies

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Abstract. Elevated levels of endogenous glucocorticoids (GCs) or prolonged treatment with high doses of dexamethasone (DEX) or other GCs preparations are frequently associated with psychosis as well as cognitive deficits, such as the impairment of memory and learning. GCs potentiate stress or ischemia-induced accumulation of excitatory amino acids in the extracellular space of hippocampus. The antagonism of glutamate receptors may potentially improve safety profile of therapy with GCs. The purpose of the present study was to investigate the effect of dizocilpine maleate (MK-801), non-competitive NMDA glutamate receptor antagonist, on neurotoxic effect of the prolonged treatment with the high dose of DEX. The results showed that DEX (120 mg/kg/day for 7 days) impaired the long-term memory and the motor coordination, reduced the body weight and induced the lethality of mice. The morphological and ultrastructural study have confirmed damage to hippocampal neurons especially in the CA3 region after the prolonged treatment with DEX alone. Damaged pyramidal neurons showed robust changes in the shape of the nucleus and cytoplasm condensation. MK-801 alone (at non-toxic dose of 0.3 mg/kg/day), changed neither the behavior of mice nor morphology of the hippocampal neurons. However, it did not prevent the neurotoxic effects of DEX. On the contrary, it intensified DEX-induced neurotoxicity.

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Key words: dizocilpine- MK-801, dexamethasone, long-term memory, motor coordination, lethality, body weight, morphological and ultrastructural study, mice

INTRODUCTION

Glucocorticoids (GCs) are often used therapeutically for their potent anti-inflammatory and immunosuppressive properties in the treatment of allergic, rheumatologic, neurological and autoimmune diseases. The most common cause of hypercortisolemia is the excessive administration of GCs as anti-inflammatory agents (Martignoni et al. 1992). Treatment with high doses of dexamethasone (DEX) (Adams et al. 1996, Borst et al. 2004, Byrne et al. 1997, Kreuter and Altmeyer 2005, Stuart et al. 2005, van der Meulen et al. 2000) or other GCs preparations, and the cases of acute or prolonged intoxications with GCs (Naumovski et al. 2003, Norra et al. 2006) can result in psychoses ("steroid psychoses"), as well as cognitive disturbances including difficulties in attention and concentration, loss of memory and impaired logical thinking (Young et al. 1999).

More recently, research has focused on observations that excessive circulating levels of GCs are frequently associated with cognitive impairment in several human pathologies, including Huntington`s disease, dementia of Alzheimer`s type, depression or psychosis (Brown et al. 1999, Ismail et al. 1995, Kiraly et al. 1997, Wolkowitz and Reus 1999, Wolkowitz et al. 1997) as well as in Cushing`s syndrome patients that suffer from depression or mania (Brown et al. 1999, Kiraly et al. 1997).

GCs, such as cortisol, are released by adrenal cortex in response to a wide range of stressors. Elevated levels of endogenous GCs can damage the brain (Brown et al. 1999, Haynes et al. 2001, Kiraly et al. 1997, Seckl and Olsson 1995), especially the hippocampus which plays an important role in memory, mood and behavior (Brown et al. 1999, Haynes et al. 2001, Newcomer et al. 1999, Wolkowitz et al. 1997). The hippocampus has the highest concentration of GC receptors in the brain and is especially vulnerable to dysfunction and degeneration in disorders of old age, including Alzheimer´s disease and depression. Moreover, it is very sensitive to many types of neurological insults, such as seizures, antimetabolite exposure, hypoxia-ischemia and exposure to various neurotoxins (Behl et al.1997, Flavin 1996).

Chronic stress or prolonged exposure to high levels of corticosterone induce dendritic atrophy in the hippocampal or striatal neurons (Brown et al. 1999, Haynes et al. 2001, Morita et al. 1999, Watanabe et al. 1992) although trophic effects of corticosteroids were also reported (McEwen et al. 1992).

It was shown that besides the participation in normal neuronal transmission or learning and memory processes, the excitatory amino acids (EAA) play a major role in various neurodegenerative diseases with cognitive dysfunction (Estupina et al. 1996, Feldman and Weidenfeld 1997, Fonnum et al. 1995, Olney and Farber 1995a,b).

A similar alterations were also noted following the administration of DEX which induced mood disorders including psychosis in some patients (Adams et al. 1996, Borst et al. 2004, Byrne et al. 1997, Ismail et al. 1995, Kiraly et al. 1997, Kreuter and Altmeyer 2005, van der Meulen et al. 2000, Stuart et al. 2005) and neuronal damage after acute as well as prolonged administration (Haynes et al. 2001, Morita et al. 1999). Prolonged exposure to DEX evoked neuronal death in layer CA3 of the hippocampus and it also enhanced necrotic death of C6 glioma cells in rats (Haynes et al. 2001, Morita et al. 1999, Sekita-Krzak et al.1999, 2003). Moreover, DEX aggravates ischemic neuronal damage by causing glutamate to accumulate in the extracellular space (Chen et al. 1998, Fuchs and Flűge 1998). DEX may also increase glutamate release, decrease its uptake or up-regulate glutamate receptor expression (Flavin 1996, Meetze et al. 1992).

The excitatory neurotransmitter glutamate has been pathogenetically linked to cell death in acute neurodegenerative disorders in humans as stroke or traumatic brain injury (Ikonomidou et al.1999, 2000, Qiang et al. 2002, Woźniak et al. 1996). Interest in potential clinical uses of non-competitive NMDA receptor antagonists grew when it was found that they all had potent neuroprotective properties following brain hypoxia and ischemia. Some authors suggest the neuroprotective effect of NMDA glutamate receptor antagonists in some acute or chronic neurodegenerative diseases, such as Parkinson´s disease, other neurogenic motor diseases and cerebrovascular, gerontopsychiatric diseases (Danysz et al. 1997, Danysz and Parsons 2003, Parsons et al. 1999) or after traumatic brain injury or cerebral ischemia (Bertorelli et al. 1998, Gorgulu et al. 2000).

Our previous study (Danilczuk et al. 2005) has shown that (+)- MK-801 (dizocilpine maleate) and memantine (both non-competetive NMDA receptor antagonists) used at the low doses did not prevent the DEX- induced toxicity but paradoxically aggravated this impairment.

Considering that many authors indicated that the higher doses of NMDA receptor antagonists could have a neuroprotective effect (Bertorelli et al. 1998, Ikonomidou et al. 2000, Misztal et al. 1996) the aim of this study was

to assess the influence of chronic treatment with MK-801 (0.3 mg/kg/day) on neurotoxicity induced by DEX (120 mg/kg/day). For this purpose, the behavioral and morphological effects of the chronic treatment with DEX alone or combined with MK-801 were studied in Albino mice.

METHODS

Subjects

Male Albino Swiss mice (initial weight 22–26 g) were used in the experiments. They were housed 12 per cage at the temperature of 20°C ± 2°C in natural light-dark cycle. The animals were allowed free access to standard laboratory food (LSM, Motycz, Poland) and tap water. All experimental procedures were performed between 8.00 A.M. and 14.00 P.M.

The procedures were conducted according to NIH Animal Care and Use Committee guidelines, and approved by the Ethics Committee of Medical University of Lublin.

Drugs

The following drugs were used: dexamethasone (DEX) (Dexaven, Jelfa, Poland) and (+)-MK-801 (dizocilpine maleate, Sigma, USA). MK-801 was dissolved in distilled water and injected 30 min before administration of Dexaven.

For assessment of the behavior, a "chimney" test, passive avoidance acquisition and retention testing were carried out. The changes in morphology and ultrastructure of hippocampal neurons were analysed in all treated animals. For this purpose, the drugs were injected intraperitoneally (i.p.), once a day, alone or in combination for 7 days. The control group received saline injections. The behavioral retests were performed 24 or 48 h after the last injection of DEX. Body weight and lethality were controlled every day during the 15 days of the experiment.

"Chimney" test

The effects of the chronic treatment with DEX alone or combined with NMDA antagonist on motor performance were evaluated with the "chimney" test (Boissier et al. 1960). The animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length). Motor impairment was indicated by the inability of mice to climb backwards up the tube within 60 s. The mice were pretrained 24 h before the treatment and those which were unable to perform the test were rejected from experimental groups.

Passive avoidance acquisition and retention testing

The step-through passive avoidance task is regarded as a measure of long-term memory acquisition (Venault et al. 1986). The mice were placed in an illuminated box (10 × 13 × 15 cm) connected to a larger (25 × 20 × 15 cm) dark compartment equipped with an electric grid floor. In this test, entry into the dark compartment was punished by an electric footshock (0.6 mA for 2 s) for acquisition facilitation. The mice that did not enter the dark compartment within 60 s were excluded from the experiment. On the following day (24 h later), the same animals were again placed in the illuminated box and those avoiding the dark compartment for longer than 180 s were regarded as remembering the task. Retention was evaluated as the mean time (in seconds) required to enter the dark compartment.

Histological study

For histological studies 48 h after the last injection of DEX or saline all animals were anesthetized with pentobarbital (Nembutal 180 mg/kg) and perfused with 0.9% NaCl with heparin, followed by 4% formaldehyde in phosphate butter (pH 7.4). Following decapitation, brains were removed from the skull and postfixed in the same fixative at 4°C for at least 24 h. Specimens were then dehydrated in graded ethanol solutions and embedded in paraffin. Six-µm thick paraffin slices were serially cut in the frontal plane. For histological analysis selected paraffin-embedded tissue slices were stained with cresyl violet and assessed using a light microscope ((LABAPHOT-2 STAND, Nikon, USA). We examined the morphology of neurons in the dorsal hippocampus of both hemispheres.

Ultrastructural study in the CA3 region

For ultrastructural studies brains were perfusion-fixed in 4% glutaraldehyde. Next, after being dehydrated, tissue material was embedded in Epon 812 (Electron Microscopy Sciences, USA). Semi-thin sections were stained with methylene blue and examined

in order to dissect the CA3 region of the hippocampal formation using a light microscope. The preparations were observed using a transmission electron microscope (TESLA BS 500, Czech Republics).

Quantitative analysis and statistics

The results of the experiments are expressed as the mean ± SEM. The data of behavioral tests were analyzed by Mann–Whitney *U* test, whereas body weight data were assessed by one-way analysis of variance (ANOVA) and Tukey–Kramer Multiple Comparisons post test. Lethality was assessed with Fisher`s Exact Test.

Quantitative analysis of morphological changes was carried out by counting the number of damaged neurons in the CA3 region (in cresyl violet-stained sections) using a computer analysis system for histological pictures (LABAPHOT-2 STAND, Nikon, USA). Cells with round, clear nuclei and visible nucleoli were considered undamaged, while neurons with dark perikarya were considered damaged. Cell counts were carried out within one 40× microscopic field in the pyramidal cell layer (from the point directly ventral to the most lateral extension of the upper limb of the dentate granule cell layer). The percentage of damaged neurons in the CA3 region was counted in all groups of animals.

Thirty sections per each animal were used for the counting procedure.The statistical significance of the differences between groups was assessed by one-way analysis of variance (ANOVA) and Student–Neuman–Keuls multiple comparisons post test. Statistically, significant differences were designated by $P<0.05$.

RESULTS

Motor coordination ("chimney" test)

As shown on Fig. 1, DEX given for 7 days at the dose of 120 mg/kg/day significantly increased the time of climbing in the "chimney" test (by about 100%) (Mann–Whitney *U* test, $P<0.02$).

MK-801 at the dose 0.3 mg/kg/day, given alone did not change the motor coordination but in combination with DEX it significantly (by about 100%) potentiated the prolongation of the time climbing induced by DEX (Mann–Whitney *U* test, $P<0.01$).

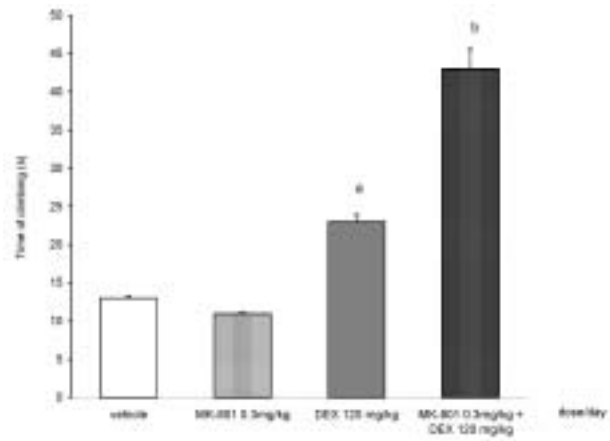

Fig. 1. The effect of prolonged treatment with dizocilpine (MK-801) on the motor coordination impaired by dexamethasone (DEX) ("chimney" test). MK-801 (0.3 mg/kg/day) was injected once daily for 7 days, 30 min before DEX (120 mg/kg/day). The last doses were given 24 h before the test. The date are presented as the mean ± standard error of mean (SEM); [a]$P<0.02$ vs. vehicle, [b]$P<0.01$ vs. DEX alone treated mice, Mann–Whitney *U* test, n=6–11.

Long-term memory task (the passive avoidance acquisition and retention testing)

DEX given alone for 7 days significantly decreased the retention time of mice in the memory task (by about 35%) in comparison with the control group (Mann–Whitney *U* test, $P<0.01$) (Table I).

Table I

The effect of the prolonged treatment with dizocilpine (MK-801) on long-term memory acquisition (passive avoidance acquisition and retention testing) impaired by dexamethasone (DEX)

DRUGS (dose/24 h)	RETENTION TIME (S) Means ± SEM
Vehicle	169.9 ± 5.4
MK-801 0.3 mg/kg	150.0 ± 4.5
DEX 120 mg/kg	110.6 ± 19.3[a]
MK-801 0.3 mg/kg + DEX 120 mg/kg	78.8 ± 23.2

DEX was administered once daily for 7 days, the last dose was given 48 h before the test. MK-801 was given 30 min before the injection of DEX. [a]$P<0.01$ DEX vs. vehicle; MK-801+DEX vs. DEX – not significant, $P<0.2$, Mann–Whitney *U* test, n=6–11.

Table II

The effect of the prolonged treatment with dizocilpine (MK-801) on the dexamethasone (DEX) lethality in mice

DRUGS (dose/24 h)	The number of dead mice/total number of mice days of experiment				
	3	7	10	12	15
Vehicle	0/12	0/12	0/12	0/12	0/12
MK-801 0.3 mg/kg	0/12	0/12	0/12	0/12	0/12
DEX 120 mg/kg	0/12	2/12	8/12[a]	10/12[a]	12/12[a]
MK-801 0.3 mg/kg+DEX 120 mg/kg	0/12	6/12[b]	10/12	12/12	

The drugs were administered for 15 days. MK-801 was given each day 30 min before the injection of DEX. [a]P<0.001 vs. vehicle, [b]P<0.05 vs. DEX treated alone. Fisher`s Exact Test. n=6–12

MK-801 tended to aggravate (MK-801+DEX vs. DEX, P<0.2) the acquisition of memory in mice exposed to the treatment with DEX, although MK-801 given alone did not influence the retention time (Table I).

Lethality

As presented in Table II, DEX (120 mg/kg/day) given alone significantly evoked the mortality in mice during the 15 days of experiment, especially between 10 day (8/12) and 15 day (12/12) (Fisher`s Exact Test, $P$<0.001). MK-801 (0.3 mg/kg/day) increased the DEX lethality of mice after 7 days (6/12, Fisher`s Exact Test, P<0.03) although MK-801 given alone did not induce the mortality of mice.

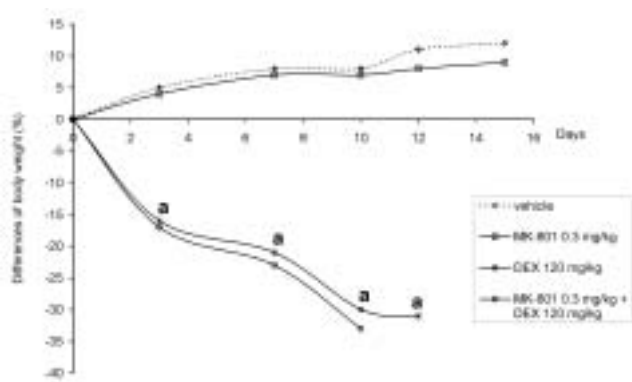

Fig. 2. The effect of prolonged treatment with dizocilpine (MK-801) on dexamethasone (DEX)-induced reduction of the body weight gain in mice. The body weight was monitored in different days of observation, depending on the different time of the mice's survival in the experiment.
The drugs were administered once daily: MK-801 (0.3 mg/kg/day) – 30 min before DEX (120 mg/kg/day). [a]P<0.001 vs. vehicle, One-way analysis of variance (ANOVA) and Tukey–Kramer post test, n=6–12.

Body weight changes

After 15 days of observation, the increase by about 10% of initial body weight in the control mice body weight gain was noted (Fig. 2). DEX (120 mg/kg/day) given alone for 12 days significantly decreased the body weight gain in comparison with initial body weight or with the control group by about 30% (One-way ANOVA, $F_{3,36}$=14.632, P<0.0001). Neither given alone nor co-treated with DEX, MK-801 (0.3 mg/kg/day) modified the body weight of mice in comparison with the control group or with DEX alone.

Histological study

Light microscopy examination of cresyl violet stained sections from the control group revealed the regular structure of the hippocampus. The nuclei of pyramidal neurons in CA1–CA4 regions were clear, round or oval in shape with distinct nucleoli. In the CA3 region they were arranged in 3 to 4 layers (Fig. 3). Seven-day long administration of DEX caused morphological changes in hippocampal neurons. DEX produced damage to hippocampal neurons especially in the CA3 region. Damaged pyramidal neurons in the CA3 region showed robust changes in the shape of the nucleus and cytoplasm condensation. These neurons were dark and shrunken.

After 7-day administration of MK-801 slight morphological changes of hippocampal neurons were observed. Single neurons in the CA3 region showed increased stainability without cell body shrinkage. Other hippocampal neurons were undamaged.

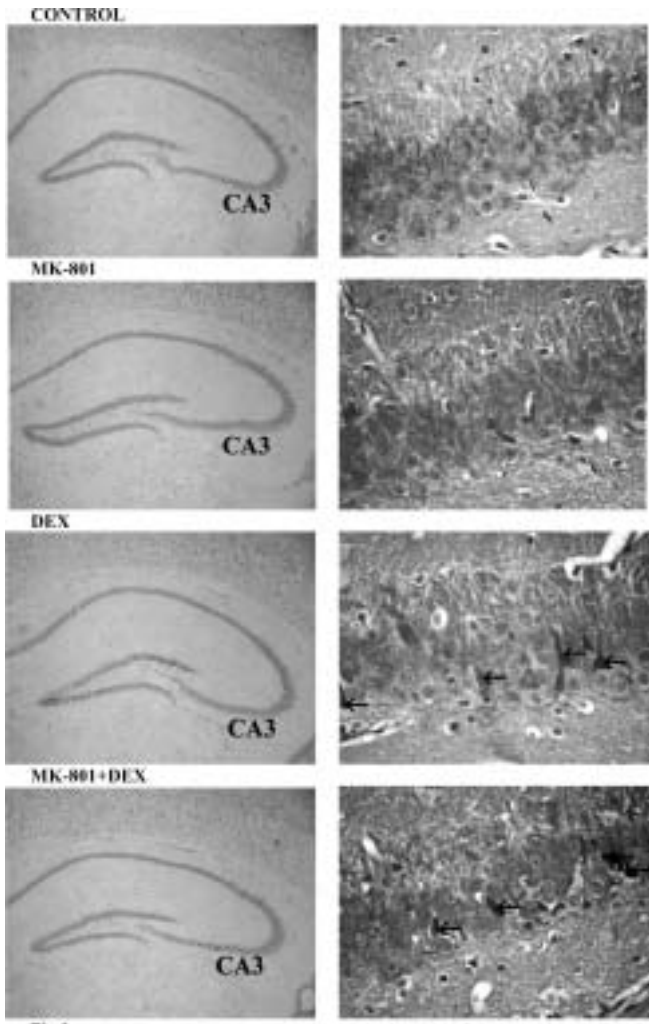

Fig. 3. Coronal sections of the dorsal hippocampus from control animal (Control); MK-801 treated animal (MK-801); dexamethasone treated animal (DEX) and MK-801+dexamethasone treated animal (MK-801+DEX). The left photomicrographs show small magnifications of the dorsal hippocampus in respective groups. Cresyl violet staining. Magnification × 100. The right photomicrographs show high magnifications of the CA3 region in respective groups. Note morphological damage of neurons after administration of DEX and slightly increased damage after administration of MK-801 with DEX (arrows show shrunken, dark neurons). Cresyl violet staining. Magnification × 400. MK-801 (0.3 mg/kg/day) was administered once daily 30 min before DEX (120 mg/kg/day) for 7 days.

In the group of animals receiving MK-801 along with DEX examinations revealed a significantly higher degree of morphological damage to CA3 pyramidal neurons in comparison with DEX alone. The number of nerve cells in the CA3 region showing morphological damage increased in comparison with the animals receiving DEX alone.

Quantitative analysis

In the control group the mean percentage of damaged neurons was 0.8%. In the experimental groups the number of damaged neurons were 3.2% after MK-801 and 9.7% after DEX (Fig. 4). This increase in the number of damaged cells was not statistically significant as compared to the control group.

In the group receiving MK-801 along with DEX the number of damaged neurons was 18.1%. The effect was statistically significant in comparison with that caused by DEX alone (One way ANOVA, $F_{3,34}$=7.903, P<0.0004 and Student–Neuman–Keuls multiple comparison post test, P<0.05).

Ultrastructural study in the CA3 region

Electron microscopy revealed that prolonged treatment with MK-801 causes slight morphological changes of pyramidal neurons in the shape of dilatation of endoplasmic reticulum cisternae in the cell cytoplasm (Fig. 5).

Seven-day administration of DEX caused profound ultrastructural changes in some pyramidal neurons in

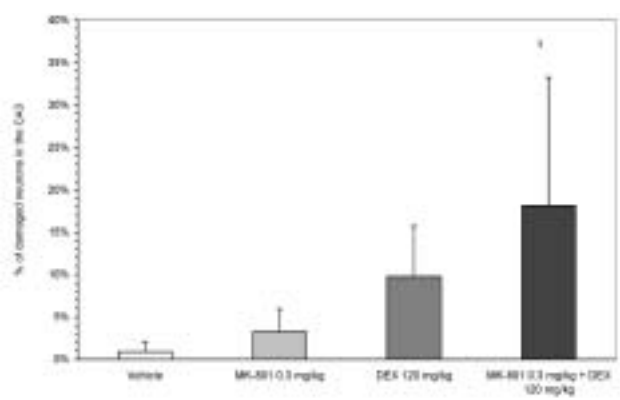

Fig. 4. Percentage of damaged neurons in the CA3 region of hippocampus. MK-801 (0.3 mg/kg/day) was given 30 minutes before DEX (120 mg/kg/day) for 7 days. One-way analysis of variance (ANOVA) and Student–Newman–Keuls multiple comparisons post test, ^{a}P<0.05 vs. DEX.

the CA3 region. Damaged pyramidal neurons in the CA3 region were dark and irregular in shape and possessed dark, irregular nuclei, as a result of chromatin and cytoplasm condensation. In the cytoplasm the areas rich in clumped ribosomes were separated by clear spaces representing the cisternae of endoplasmic reticulum. Ribosomes, endoplasmic reticulum and mitochondria showed morphological damage. Shrunken neurons were surrounded by swollen processes of glial cells.

In the group of animals receiving MK-801 along with DEX, pyramidal neurons in the CA3 region revealed more severe damage, as concluded from the appearance of the ribosomes, endoplasmic reticulum and mitochondria which showed higher degree of disintegration in comparison with cell organelles observed after administration of DEX alone.

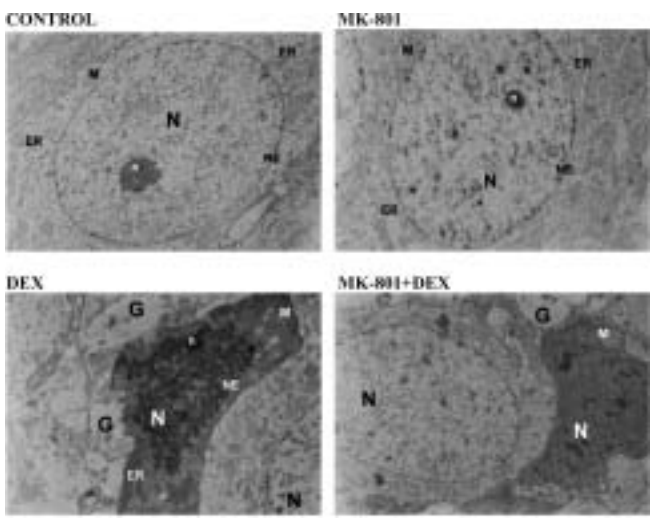

Fig. 5. Electron micrographs of the pyramidal neurons in the CA3 region of the hippocampus in respective groups. Control – (N) nucleus; (n) nucleolus; (NE) nuclear envelope; (M) mitochondrium; (ER) endoplasmic reticulum. Magnification × 6000; MK-801 – the dilatation of endoplasmic reticulum cisternae. Magnification × 6000; DEX – shrunken and dark neuron surrounded by swollen processes of glial cells; (N) nucleus; (n) nucleolus; (NE) nuclear envelope; (M) mitochondrium; (ER) endoplasmic reticulum; (G) swollen processes of glial cells. Magnification × 6000; MK-801+DEX – the neurons with various degree of morphological damage; the dark neuron shows strong condensation of the nucleus and the cytoplasm and morphologically injuried mitochondria; (N) nucleus; (M) mitochondrium; (G) swollen processes of glial cells. Magnification × 6000. MK-801 (0.3 mg/kg/day) was injected 30 minutes before DEX (120 mg/kg/day) for 7 days.

DISCUSSION

The results of the present study indicate that prolonged (for 7 days) administration of DEX at a dose of 120 mg/kg/day significantly decreased the retention time of mice in memory task and impaired the motor coordination in "chimney" test. Moreover, administration of DEX at that dose for longer time (up to 15 days), significantly reduced the body weight and caused the lethality of mice.

We have also shown that 7 day DEX administration in a dose of 120 mg/kg damaged hippocampal pyramidal neurons especially in the CA3 region. Damaged neurons showed robust changes in the shape of the nucleus and demonstrated cytoplasm condensation. Dark and shrunken neurons were surrounded by swollen processes of glial cells, indicating a development of reactive gliosis. Ultrastructural examination demonstrated the damage to ribosomes, endoplasmic reticulum and mitochondria and confirmed light microscopy observations on the subfield-specific neurotoxic effect of DEX in hippocampal formation.

We have found that MK-801 at the dose of 0.3 mg/kg/day did not change either the behavior, or body weight in comparison with the control group. Minor damage to pyramidal neurons observed in about 3% of cell population and slightly altered ultrastructure of the CA3 pyramidal neurons as concluded from the shape of dilatation of endoplasmic reticulum cisternae may indicate only the slight side effect following MK-801 treatment.

MK-801 at the dose of 0.3 mg/kg/day administered to the animals treated with DEX was not effective in counteracting behavioral impairment and the lethality induced by DEX administration; on the contrary, the potentiation of these changes was observed. This was concordant with the intensity of degenerative changes evaluated at the light microscopy level confirmed by the alterations in the ultrastructure of pyramidal cells. All these results indicate that MK-801 at the dose of 0.3 mg/kg/day does not protect against the neurotoxic effect of DEX. The behavioral results obtained in this study are consistent with our previous study (Danilczuk et al. 2005) when MK-801 given at the lower doses (25 or 50 μg/kg/day) as a co-treatment with DEX (80 mg/kg/day) significantly disturbed the motor coordination and acquisition of memory and also reduced the body weight and increased the lethality caused by DEX.

The results of other authors indicate that GCs impair the spatial memory in rats (De Quervain et al. 1998, Endo et al. 1996). Several days of exposure to cortisol at the doses causing plasma concentrations associated with physical and psychological stress in human, can reversibly decrease the specific elements of memory performance in otherwise healthy humans (Keenan et al. 1996, Newcomer et al. 1999, Wolkowitz et al. 1997). In rats DEX administered at the single dose or chronically (at low doses) reduced the locomotor activity of rats (Danilczuk et al. 2001, Haynes et al. 2001).

The apparent association between neurological disturbance, memory performance, circulating levels of adrenal corticosteroids and histological changes in the brain showed that GCs are toxic to pyramidal neurons of the hippocampus, particularly in the CA3 subfield (Haynes et al. 2001, Morita et al. 1999, Sekita-Krzak et al. 1999, 2003) and in the striatum (Griffiths et al. 2000, Mitchell et al. 1998, Morita et al. 1999) what may be a cause of many neurological disorders.

In the studies upon subfield-specificity of DEX-induced neuronal loss in the hippocampus Sousa and others (1999) demonstrated that the most severely affected region is the CA3. The CA3 pyramidal neurons display type II GCs receptor binding sites, and that is why DEX – a highly selective type II GCs receptor ligand – affects them.

The mechanisms underlying GCs-induced neuronal damage have been extensively discussed. There are numerous inhibitory effects of GCs that are site-preferential to the hippocampus, including inhibition of glucose transport into hippocampal neurons and glia at the high concentrations of GCs, involution of dendric processes of hippocampal neurons and inhibition of long-term potentiation, as revealed in experiments using stress paradigms. Moreover, preincubation of neuron-glial culture with nanomolar concentrations of DEX resulted in a dose-dependent increase of injury (Flavin 1996). Combined glutamate receptor antagonist application counteracted this deleterious effect, suggesting that DEX may increase glutamate release, decrease uptake and upregulate glutamate receptor expression (Flavin 1996, Meetze et al. 1992).

An activation of NMDA receptors (NMDAR) by high concentrations of glutamate may be decisive for induction of degenerative changes in nerve cells under the influence of GCs which can further contribute to neuronal damage triggered by glutamate by reducing the antioxidant enzyme capacity in brain, thus making neurons more susceptible to free radicals generated by excitotoxic stimuli (McIntosh and Sapolsky 1996).

Due to NMDAR antagonists ability to block excitotoxic actions of glutamate, MK-801 could prevent the neuronal degeneration induced by GCs, and neurotoxic effects of DEX in particular. In the past years, neuroprotective effect of NMDAR antagonist has been demonstrated (Danysz and Parsons 2003, Danysz et al. 1997, Ellison 1995, Muir and Lees 1995, Parsons et al. 1999), although undesirable side-effects of NMDA-antagonists have been reported. In addition to their apparent psychotomimetic properties and neurological disturbances, such as ataxia or increase in muscle ton (Farber et al. 1995, Kornhuber and Weller 1997, Zajączkowski et al. 1997), or the impairment of memory (Mondadori and Weiskrantz 1993, Roesler et al. 1999, Roozendaal and McGaugh 1996, Zajączkowski et al. 1997), these compounds were found to have neurotoxic effects themselves (Qiang et al. 2002).

Zajączkowski and coauthors (2000) demonstrated that MK-801 at the high dose (5 mg/kg/day) evoked recumbence, hypothermia and loss of the body weight in female rats after 3–7 days administration of the drug. Despite that, there were no differences found in performance of avoidance reaction between saline and MK-801 treated animals trained for 10–40 days after drug administration.

In our study, after 7 days of MK-801 (0.3 mg/kg/day) administration the memory performance was not disturbed either. No changes in the motor coordination or body weight nor mortality of mice were observed. Surprisingly, it did not have any neuroprotective effect on neurotoxicity induced by DEX, on the contrary, it intensified DEX-induced neurotoxic effects. These results are compatible with the findings of Ikonomidou and colleagues (2000). The authors have demonstrated that MK-801 given alone at the dose of 0.3 mg/kg/day for over 28 days did not cause neurological impairment or mortality (Ikonomidou et al. 2000) but exacerbated slowly the progressing neurodegeneration in the striatum, produced by neurotoxine 3-nitropropionate (3NP), and significantly increased the mortality of rats as compared with treatment with 3NP or vehicle. Other NMDA – antagonists (memantine and CPP), but not AMPA – antagonists (NBQX or MPQX), induced similar neurotoxic effect (Ikonomidou et al. 2000).

There are several hypotheses on the mechanisms of neurotoxicity of NMDA-antagonists. Ikonomidou and others (2000) proposed that decrease of intracellular Ca^{2+} concentration caused by blockade of Ca^{2+} permeable NMDA channels may be a mechanism contributing to enhancement of neuronal death by NMDA antagonists. Accordingly, when NMDA receptors are selectively blocked by antagonists, glutamate will activate AMPA/kainate receptors, as long as imbalance in NMDA system continues and promotes neuronal death. It is also speculated that glutamate acting only via NMDA (but not AMPA) receptors may serve a trophic function cooperating with e.g. activity-dependent brain-derived neurotrophic factor (BDNF) neurotrophin TrkB receptor which mediates prosurvival signals and regulates neuronal plasticity (Elmariah et al. 2004, Skup et al. 2002, Suzuki et al. 2005) As suggested by Qiang and coauthors (2002) NMDA antagonists produce neurodegeneration by reducing GABAergic inhibition, as concluded in their studies on the posterior cingulate cortex/retrosplenial cortex.

Moreover, it has been reported that DEX-induced apoptosis of the subpopulation of striatopallidal neurons is virtually identical to that caused by NMDA receptor antagonist PCP (Mitchell et al. 1998). The authors suggest that PCP could induce striatal cells death *via* a corticoid-dependent mechanism (Mitchell et al. 1998). It was supported by the observation, that co-injection of the GCs receptor antagonist, RU 38486, markedly attenuated the levels of PCP-induced striatal cell death (Haynes et al. 2001).

On the basis of our and other studies, it can be suggested that the injures in some brain regions induced by DEX may evoke higher vulnerability of these regions to a damaging effect of NMDA antagonists. Further studies are needed to explain the mechanism of the observed interaction between DEX and NMDA receptor antagonists.

CONCLUSIONS

The findings of the present study provide experimental evidence that prolonged treatment with DEX, at the high dose (120 mg/kg), disturbed the motor coordination and acquisition of memory, reduced the body weight and induced the lethality of mice and caused

a damage the hippocampal pyramidal neurons, especially in the CA3 region. Dizocilpine (MK-801), noncompetitive NMDA receptor antagonist, given at nontoxic dose (0.3 mg/kg/day) did not prevent the DEX-induced neurotoxicity, but on the contrary, it potentiated the neurotoxic effect of DEX.

It is suggested that the injures induced by DEX in some brain regions may evoke higher vulnerability of these regions to a damaging effect of NMDA antagonists.

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Received 2 February 2006, accepted 25 September 2006