STRAIN DIFFERENCES IN THE EFFECT OF LONG-TERM TREATMENT WITH MELATONIN ON KAINIC ACID-INDUCED STATUS EPILEPTICUS, OXIDATIVE STRESS AND THE EXPRESSION OF HEAT SHOCK PROTEINS

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ABSTRACT

The present study compared the effects of subchronic treatment with melatonin administered via subcutaneous osmotic minipumps for 14 days (10 mg/kg per day) on kainic acivd (KA)-induced status epilepticus, oxidative stress and expression of heat shock protein (HSP) 70 in the frontal cortex and hippocampus between normotensive Wistar rats and spontaneously hypertensive rats (SHRs). SHRs showed increased lipid peroxidation (LP) in the cortex and hippocampus and decreased cytosolic superoxide dismutase (SOD/CuZn) production in the cortex compared to Wistar rats. Long-lasting seizures induced by KA (12 mg/kg, i.p.) were accompanied by increased LP and expression of HSP 70 in the hippocampus of the two strains and increased SOD/CuZn production in the frontal cortex of SHRs. Pretreatment with melatonin failed to supress the KA-induced SE in the two strains though the latency for seizure onset was significantly increased in SHRs. The increased LP induced by KA in the hippocampus was attenuated by melatonin pretreatment both in Wistar rats and SHRs. The increase of SOD/CuZn and mitochondrial SOD/Mn production was strain- and area-specific in melatonin- KA treated groups. Melatonin prevented the KA-induced increased expression of HSP 70 in the hippocampus of KA-treated Wistar rats. The study, suggest that the potential efficacy of melatonin pretreatment on SE-induced oxidative stress and neurotoxicity is strain- and area-specific.

Key words: *Kainic acid; melatonin; oxidative stress; heat shock protein; spontaneously hypertensive rats; Wistar rats.*

Introduction

Epilepsy has been described as a condition of excessive neuronal discharge associated with or resulting from oxidative stress (Shin et al., 2011; Waldbaum and Patel, 2010). Epileptic biomarkers, such as catalytical antioxidants and oxidative products in neuronal tissues have been monitored for evaluation of the degree of epileptic pathogenesis. The neurotoxin kainic acid (KA) triggers neuropathologic cellular changes in the hippocampus characterized by an overloading of intracellular calcium, mitochondrial membrane rupturing, activation of intracellular enzyme cascades, including the nitric oxide synthase and increased levels of free radicals/reactive oxygen species (ROS) from the mitochondrial intermembrane space into the cytosol (Srivastava et al., 2008). The formation of free radicals results in an extensive lipid peroxidation, which damage cellular organelles and membranes, and finally leads to cell death. Thus, oxidative stress resulting from excitotoxicity is suggested to play a critical role in epileptic brain damage (Bondy and Lee, 1993). Harmful changes in cells, including calcium influx and generation of ROS induce or suppress the expression of genes in neurons thereby influencing synthesis of proteins (Rajdev and Sharp, 2000). The expression of stress proteins, referred to members of heat shock proteins (HSP) is caused by a variety of injurious stimuli in the brain and is also considered as an appropriate marker of exitoxicity. Several studies suggested that there exist a close relationship between ROS and the expression of members of the Hsp70 family (Ambrosio et al., 1995; Kukreja et al., 1994; Lee J. Y.

and Corry, 1998). In addition, the increased expression of HSP 70 was seen after KA-induced SE in rat brain (Gupta and Briyal, 2006).

The disturbance in the levels of the antioxidant enzymes is a crucial step involved in dysregulation of physiological processes implicated in the pathogenesis of arterial hypertension (Harris, 1992). Experimental data demonstrate that the direction of changes in the activity of antioxidant system strongly depends on the severity of oxidative stress. In this regard, moderate levels of ROS production are able to enhance the activity of the defense system by stimulation of different antioxidant enzymes (Vogt, 1998). However, when the oxidative load exceeds the defense potential, the adaptive response of the antioxidant system could be disturbed (Csonka et al., 2000). It has been reported that the expression of antioxidant enzymes is increased in the myocardium of spontaneously hypertensive rats (SHRs) following the induction of oxidative stress (Csonka et al., 2000). However, the reduction of the defense antioxidant enzymatic activities in SHRs compared to normotensive Wistar Kyoto rats indicates a disturbance of the defence system as a sequence of the enhanced oxidative stress in the SHR model (Polizio and Peña, 2005). Currently, number of protective approaches with potential antioxidants has been applied to improve hypertension (Levonen et al., 2008; Schiffrin et al., 2010). In addition to a widely accepted experimental model of essential hypertension, SHRs could be considered as a tool for studing the link between hypertension and epilepsy (Greenwood et al., 1989; Scorza et al., 2005; Tchekalarova, 2010, 2011). Furthermore, substantial data support the view point that some of the physiological and biochemical markers of epilepsy are also evident in naive SHRs. Thus, hippocampal neuropathology, including neuronal loss, mostly in the CA_1 subfield, and astrocyte reactivity has been reported in intact adult SHRs (Pietranera et al., 2006; Sabbatini et al., 2000). Vorobyov and co-authors (2011) found that the EEG spectral profiles are similar in SHRs suffering from congenital hypertension and in KA-treated normotensive rats. In this regard, we have shown that naive and epileptic SHRs exhibit similar anxiety level associated with low level of serotonin and dopamine in the hippocampus (Tchekalarova et al., 2011). Moreover, the naïve SHRs were characterized with abnormal behavioral responses and biochemical parameters, which are also characteristic for epileptic rats (Tchekalarova et al., 2011).

Clinical evidence has revealed that melatonin is implicated in epilepsy, it is able to reduce the spiking activity and seizure frequency in patients with intractable epilepsy (Anton-Tay, 1974; Molina-Carballo et al., 1997) and it is detected in high levels during the postictal period (Bazil et al., 2000). Melatonin posses a low toxicity and may be used for seizure control in conjunction with anti-seizure medications (Rufo-Campos et al., 2002). Melanonin has been also characterized as a potent anticonvulsant in a number of seizure models in rodents, including acute seizure tests (Albertson et al., 1981; Borowicz et al., 1999; Lapin et al., 1998; Yamamoto and Tang, 1996) and models of epilepsy (Albertson et al., 1981; Mevissen and Ebert, 1998; Tchekalarova et al., 2013). It has been shown to exert neuroprotective effects against neuronal damage in animal models of neurotoxicity i.e. stroke and traumatic brain injury (Chung, 2003) as well as toxic quinones and oxidative stress produced by catecholamines (Hirata et al., 1998). Single injection of melatonin in rats before and during the KA- or pilocarpine-induced status epilepticus (SE) has neuroprotective effect by reducing the neuronal death, supragranular mossy fiber sprouting, lipid peroxidation (LP), and microglial activation (Banach et al., 2011; de Lima et al., 2005; Guisti et al., 1996). So far, there has been accumulated a broad spectrum of in vitro and in vivo studies confirming the attitude that melatonin behaves as a free radical scavenger and potent antioxidant (reviewed in: Russel et al., 2000). Although the efficacy of a single injection of melatonin against oxidative stress and KA-induced seizures has been already studied in mice (Mohanan and Yamamoto, 2002), there is none study focused on comparative investigation of the putative role of long-term melatonin exposure against the KA-induced excitoxicity during the acute period between SHRs and normotensive Wistar rats.

The brain is particularly sensitive to attacks of ROS. The frontal cortex and the hippocampus are connected with each other through different neurotransmitter systems and have been proposed to being particularly vulnerable to KA-induced SE (Ben-Ari et al., 1980; Schwob et al., 1980; Sperk et al., 1983; Chen and Buckmaster, 2005). We hypothesized that the rate of oxidative damage during SE may vary in a region- and melatonin- specific manner in Wistar rats and SHRs. Therefore, the aim of the present investigation was to check out and compare the efficacy of subchronic melatonin exposure on KA-induced seizure severity and changes in the LP, enzymatic antioxidant defense systems and heat shock protein (HSP) 70 expression in the frontal cortex and hippocampus at 4 hour (h) following SE in normotensive Wistar rats and SHRs.

2. Materials and methods

2.1. Animals

The experiments were performed on adult male normotensive Wistar rats obtained from the animal facility of the Bulgarian Academy of Sciences and spontaneously hypertensive rats (SHRs) from the local breeding house (Medical University, Sofia). The rats weighing 180-200 g were adapted for one week under standardized laboratory conditions (12 h/12 h light/dark cycle, temperature 22±2^{o}C, 50 % relative humidity) in groups of 2 in plastic cages with soft bedding. Food and water were available ad libitum throughout the study except during the tests. The experimental protocol was in compliance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) and the experimental design was approved by the Institutional Ethics Committees of Sofia Medical University and the Institute of Neurobiology for the National Science Fund grant DTK 02/56 2009-1012.

2.2. Experimental design

The animals were divided into eight experimental groups (n=10) as follows: Group I: Naive and sham normotensive Wistar group treated with vehicle (Wis-C-veh); Group II: control+melatonin Wistar group (Wis-C-mel); Group III: Kainic acid (KA) group (Wis-KA-veh); Group IV: KA + melatonin group (Wis-KA-mel); Group V: Naive and sham spontaneously hypertensive group (SHRs-C-veh) treated with vehicle; Group VI: control+melatonin SHR group (SHRs-C-mel); Group VII: KA group (SHRs-KA-veh); Group VIII: KA + melatonin group (SHRs-KA-mel). Melatonin (Sigma-Aldrich, Bulgaria) was applied chronically for a period of two weeks via osmotic minipumps at a dose of 10 mg/kg/day. Alzet osmotic minipumps were filled with drug dissolved or vehicle (0.9% NaCl and DMSO (2:1), pumping rate 0.5 μl/h, information provided by manufacture). There are not established any differences between naive and sham rats. A method of melatonin infusion via s.c. osmotic minipumps provided constant steady-state hormonal concentrations.

2.3. Arterial blood pressure measurements

Systolic arterial blood pressure (ABP) was measured non-invasively in conscious unrestrained SHRs by a tail cuff method (Ugo Basile Blood Pressure Recorder 5800) before the start of experimental procedures The ABP value for each rat was calculated as mean of three measurements.

2.4. Kainic acid-induced status epilepticus

On the 14th day of vehicle/melatonin s.c. infusion animals from groups III, IV, VII and VIII received intraperitoneal injection of KA (Ascent Scientific, UK) at a dose of 12 mg/kg dissolved in sterile saline (0.9 % NaCl) or saline (groups I, II, V, VI) in a volume of 1 ml/kg of body weight. The protocol used to elicit KA-induced SE was based on previous studies (López-Meraz et al., 2005; Morales-Garcia et al., 2009). After injection of KA, the animals were put in individual cages and observed for 4 h to evaluate the appearance of seizures. The intensity of seizures was assessed

according to the Racine's scale (1972) consisting of six stages (0-5), which correspond to the successive developmental stages of motor seizures: (0) normal non-epileptic activity; (1) facial automatism, sniffing, scratching, wet dog shakes; (2) head nodding, staring, tremor; (3) forelimb clonus with lordotic posture; (4) rearing and continued forelimb clonus, salivation; (5) forelimb clonus and loss of posture. Latency for the onset of the first forelimb clonus with lordotic posture was also evaluated.

2.5. Biochemical experiments

Biochemical tests were conducted 4 h after KA injection. The animals were sacrificed by decapitation under a light anesthesia (CO2). Brains were quickly dissected on ice and the frontal cortex and the hippocampi were bilaterally removed. The tissue samples were frozen in liquid nitrogen, and stored at -70^{o}C before analysis.

2.4.1. Measurement of lipid peroxidation

The extent of lipid peroxidation was determined quantitatively by direct measurement of hydroperoxides in redox reactions with ferrous ions. Therefore the tissue samples were homogenized in cold 20 mM HEPES buffer (pH 7.2) and extracted with hlorophorm. The extracted lipid peroxides were assayed with LPO assay kit (Cayman Chemical Company, USA) according the instructions provided. The resulting ferric ions were detected using thiocyanate ion as the chromogen and by reading the absorbance at 500 nm. The extent of lipid peroxidation was expessed as nmol.

2.4.1. Measurement of cytosolic and mitochondrial superoxide dismutase

The tissue samples were homogenized in cold 20 mM HEPES buffer (pH 7.2) ans centrifugated at 1500 × g for 5 min, at 4 ◦C. To separate cytosolic and mitochondrial SOD, the 1500 × g supernatant was again centrifuged at 10 000 × g for 15 min, at 4 ◦C. The resulting supernatant was tested for cytosolic SOD and the pellet – for mitochondrial SOD

with SOD assay kit (Cayman Chemical Company, USAThe results were expressed as U/ml.

2.4.2. Heat shock protein

Western Blotting

Tissuess were washed once in ice-cold PBS and homogenized in 5 ml of cold 20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol and 70 mM sucrose. Protein concentration was determined by spectophotometric measuring of the homogenates at 280 nm. Equal amounts (20 mg/lane) of protein samples were run on an 12 % SDS polyacrylamide gel. The proteins were transferred onto nitrocellulose membrane and blocked with 3% bovine serum albumin in TBS-0.05% Tween. The membrane was incubated with the primary mouse anti-Hsp72 antibody μ chain (Invitrogen) 1:500, for 2 h at room temperature or overnight at 4°C. The membrane was washed 3 times with TBS-Tween and further incubated in the secondary antibody anti-mouse μ chain, raised in goat and conjugated with alkaline phosphatase (Vector Labs, USA) 1: 250. After 3 times washing in TBS-Tween the membrane was incubated in 10 ml of ABC-AmP reagent (Vector Labs, USA) for 10 min at room temperature and washed again. Than the membrane was equilibrated in TBS for substrate (pH 9.5) and incubated in substrate solution BCIP/NBT (Vector Labs, USA) at room temperature for about 30 min. After developing of appropriate density color bands the membrane was rinsed in PBS and air dried. On every SDS-PAGE one lane was extrapolated to the same standard of 20 mg of control rat brain tissue protein and all other bands on each gel were expressed relative to this standard. Blots were scanned and analyzed with the ImageJ software (V 1.42q).

2.5. Statistical analysis

The data are expressed as mean±S.E.M. The seizure severity scores following KA injection were evaluated by means of two-way ANOVA with subsequent comparison with Kruskal-Wallis test while the biochemical parameters by means of three-way ANOVA (SigmaStat® SPSS). The incidence of seizures and mortality was evaluated by Fisher's exact test. The level of statistical significance was set at 5 %.

3. Results

Control SHRs showed significantly higher ABP (181±1.45 mm Hg, $p<0.005$) in comparison with the normotensive WIS controls (134±1.45 mm Hg).

3.1. Effect of subchronic melatonin pretreatment on KA-induced seizures

As shown in Table 1, there were no behavioral differences between Wistar rats and SHRs pretreated with either vehicle or melatonin (10 mg/kg for 14 days via s.c. osmotic minipums). The systemic i.p. injection of a single excitotoxic dose of KA (12 mg/kg) led to development of progressive motor changes and SE both in Wistar rats and SHRs similarly to previously described (Tchekalarova et al., 2010). Most of the KA-treated animals were characterized with facial automatisms, wet dog shakes and head nodding (partial seizures) during the first hour of observation. During the second and the third hour following KA administration, this activity progressed to secondary generalized seizures i.e. forelimb clonus with lordotic posture followed by rearing and forelimb clonus and loss of posture. The Racine's score reached 3.8±0.32 points in Wistar rats and 3.7±0.47 points in SHRs, respectively. The latency for onset of the first clonic seizure induced by KA injection was significanty lower in SHRs-KA-veh group compared to Wistar-KA-veh group ($^{o}p=0.02$) (Table 1). Subchronic melatonin pretreatment significantly increased the latency for the onset of the first clonic seizure in SHRs (*P=0.034) (Table 1).

3.2. Effects of melatonin treatment on the level of lipid peroxidation

Lipid peroxidation as a marker of oxidative stress showed strain-dependent differences both in frontal cortex (#$p<0.001$) and hippocampus (#$p<0.001$). SHRs have higher level of lipid peroxidation in naïve and melatonin treated controls (#$p<0.001$), as well in KA- vehicle (#$p<0.001$) and KA- melatonin groups (#$p=0.066$) compared to Wistar rats in frontal cortex. KA-treatment provoked a significant increase in the hippocampal lipid peroxidation in Wistar rats (*$p=0.028$), which level was dramatically decreased even below the control level after sub-chronic melatonin treatment ($^{o}p<0.001$). KA-treated SHRs also displayed an increased oxidative stress in the hipocampi (*$p=0.008$), which was abolished by melatonin pretreatment ($^{o}p=<0.001$)(Fig.1). Similar drug-induced changes were not found in the frontal cortex.

3.3. Effects of melatonine on the citosolic superoxide dismutase (SOD Cu/Zn) activity in the frontal cortex and the hippocampus of Wis and SHRs

Naive SHRs showed lower SOD Cu/Zn level in the frontal cortex compared to respectively Wistar controls (#$p<0.001$). KA-treatment increased enzime level only in SHR's frontal cortex (*$p<0.001$). Although melatonin pretreatment abolished KA-induced SOD Cu/Zn increasement in SHR's frontal cortex ($^{o}p<0.001$), on the other hand the hormone treatment raise SOD Cu/Zn cortical level in KA-treated Wistar ($^{o}p<0.001$), and hippocampal ($^{o}p<0.001$) enzyme level in both strains as well as in naïve SHRs (*$p= 0.016$) (Fig 2).

3.4. Effects of melatonine on the mitochondrial superoxide dismutase (SOD Mn) activity in the frontal cortex and the hippocampus of Wis and SHRs

Neither strain differences nor KA-induced changes in SOD Mn were found in controls. Melatonin treatment, however showed dual effect – it decreased cortical SOD Mn level both in

naïve (#p=0.024) and KA-treated SHRs (#p<0.001), but increased the enzyme level in the hippocampi of KA-treated Wistar rats (^{o}p= 0.020) (Fig. 3).

3.5. Effects of melatonine on the heat shock protein 70 in the frontal cortex and the hippocampus of Wis and SHRs

KA-treatment increased HSP70 level in hippocampus of Wistar rats (*p<0.001) and in SHR's hippocampus (*p= 0.003), but this effect was weaker in SHRs (#p=0.001). SHR's frontal cortex remains unaffected by the neurotoxin. Melatonin treatment was able to abolish only KA-induced increase in HSP70 level in the hippocampi of Wistar rats (^{o}p=0.05) (Fig. 4).

Discussion

In the present study, the subchronic melatonin treatment increased the latency of KA-induced seizures in SHRs but not in normotensive Wistar rats. Similarly, administration of melatonin at a dose of 10 mg/kg for 60 days, starting three hours after the beginning of SE, exerted a more efficient attenuation of spontaneous reccurent seizures in SHRs than in Wistar rats because it was able to suppress the seizure activity after discontinuation of the melatonin treatment in SHRs (Tchekalarova et al., 2013; Petkova et al., submitted). We can suggest that the higher efficacy of melatonin treatment on seizure activity in SHRs than in Wistar rats might be related to simultaneous decrease of the blood pressure detected in epileptic SHRs (Petkova et al., unpublished). There are emerging experimental and clinical studies considering the close relationship between hypertension and epilepsy (Tomson et al., 1998; Hilz et al., 2002; Devinsky et al., 2004). However, melatonin failed to suppress the development of KA-induced SE both in Wistar rats and SHRs. These results are in accordance with our previous works demonstrating that the long-term melatonin treatment after KA-induced SE decreased the latency for onset of the first spontaneous reccurent seizure and attenuated the seizure frequency during the treatment period without preventing the development of chronic epileptic state in Wistar rats and SHRs (Thekalarova et al., 2013; Petkova et al., unpublished). Although the majority of data indicate anticonvulsant activity of melatonin in both animal models and in patients with epilepsy, this drug was only suggested for add-on therapy in epileptic patients with insomnia. The contradictiory literature data concerning the anticonvulsant efficacy of melatonin applied at pharmacological doses are related to its time-, age- and model-dependent effect. Thus, although a single injection of melatonin exerts an anticonvulsant activity in different seizure tests (Banach et al., 2011; De Lima et al., 2005), experimental data suggest that the time of administration, the duration of treatment and the age of the testing subjects are very important for drug efficacy (Costa-Lotufo et al., 2002; Musshoff and Speckmann, 2003). Costa-Lotufo and co-authors (2002) reported that subchronic (10-50 mg/kg, i.p. for one week in the morning and in the afternoon, respectively) but not acute melatonin treatment prevented the pilocarpine-induced seizure activity. Furthermore, acute melatonin injection (20 mg/kg, ip) was reported to be ineffective to PTZ and KA-induced seizures in rats (Xu and Stringer, 2008) whereas the same design of melatonin treatment suppressed the KA-induced seizures in mice and mitochondrial DNA damage in the mouse brain cortex (Mohanan and Yamamoto, 2002). Our treatment protocol provided constant steady-state drug concentration and the time of treatment could be neglected. Alternatively, our results suggest that the anticonvulsant efficacy of melatonin depends on the seizure model and co-morbid hypertension.

In accordance with several other studies (Dal-Pizzol, 2000; Gupta et al., 2002; Marini et al., 2004), we demonstrated that KA-induced SE is accompanied by increased LP level in hippocampus homogenate of SHRs and Wistar rats after 4 hours of acute phase of seizures. Our data confirm the suggestion that ROS are involved in the mechanism of neurotoxicity trigered by KA during the acute phase in SHRs and normotensive Wsitar rats. Moreover, literature data demonstrated that the increased brain LP could be detected also during the late periods (24-72 h after SE) (Candelario-Jalil et al., 2001; Dal-Pizzol, 2000; Kubera et al., 2004). Our results are in support of previous

finding that single melatonin injection is able to prevent the KA-induced LP augmentation in mice (Mohanan and Yamamoto, 2002). Although a large body of in vivo and in vitro evidence revealed that melatonin function as a free-radical scavenging antioxidant (reviewed in: Galano et al., 2011), in our study melatonin failed to decrease the LP level below the basal value in naive SHRs and normotensive Wistar rats. Indeed, Gönenç et al. (2005) revealed that melatonin administration alone decreased the level of LP in the hippocampus but not in the frontal cortex compared to control rats. These discrepancies could result from variations in experimental species, different melatonin doses and routes of administration.

In our study, the markers of oxidative stress, which showed an increased LP and decreased SOD Cu/Zn activity in a model of essential hypertension compared to Wistar rats indicates an enhanced oxidative stress in SHRs. These results confirm previous findings that naive SHRs are characterized by a disturbed oxidative defence system compared to Wistar Kyoto rats in physiological conditions (Polizio and Peña, 2005). The reported divergence of the defence system as a sequence of enhanced oxidative stress in the SHRs is in favor of the assumption that the levels of antioxidant enzymes are crucial for dysregulation of physiological processes implicated in the pathogenesis of arterial hypertension (Harris, 1992). SOD is one the most important antioxidant enzyme, which protects against oxidative damage by catalyzing the dismutation of superoxide anion to hydrogen peroxide, thus contributing to decreased formation of hydroxyl radical formation (Coyle ad Puttfarcken, 1993). In vertebrates, copper/zinc-containing SOD (SOD Cu/Zn) and manganese-containing (SOD Mn) are the predominant isoforms found either in the cytosol or the mitochondrial matrix, respectively. Mitohondrial disfunction and ROS localized there plays a crucial role in the mechanisms leading to neuronal cell death during epileptogenesis (Kunz, 2002). Several studies demonstrated KA-induced disturbance in the mitochondrial function in rodents (Liang and Patel, 2006; Milatovic et al., 2001). We found that KA provoked an enhancement of the cytosolic SOD Cu/Zn 4 hours after SE only in the frontal cortex of SHRs. Literature data showed that seizures and SE could alter oxidative stress by either activation or suppression of free radicals scavenging enzymes such as SOD in different brain areas (reviewed in: Devi et al., 2008). The increased cytosolic SOD activity in SHRs might reflect the higher seizure susceptibility following KA injection of SHRs compared to Wistar rats. Literature data support the presumption that seizures provoked changes in the oxidative defence system are influenced by the previous level of oxidative stress, brain area, strain used and time points detected for the direction of changes. Thus, Candelario-Jalil (2001) revelead that systemic administration of an excitotoxic dose of KA decreased the hippocampal SOD activity with respect to basal levels detected 24 h after KA application. On the other hand, the increased hippocampal LP after KA or pilocarpine-induced SE in female Wistar rats were more pronounced at 12-14 h after SE but returned to basal level in KA model or decreased in pilocarpine model 7-9 days or 75-80 days after the end of SE (Dal-Pizzol et al, 2000). Antioxidant-like melatonin increased cytosolic and mitochondrial SOD activity in the frontal cortex and the hippocampus but the efficacy of this hormone was higher in normotensive Wistar rats than SHRs. The observed decrease in the LP level and increased SOD activity after melatonin pretreatment in KA-induced SE confirm the broadly accepted assumption that melatonin function as a free-radical scavenging antioxidant and inhibitor of LP (reviewed in: Yonei et al., 2010). However, comparison of the anticonvulsant and antioxidant efficacy of melatonin in the two strains suggests lack of a direct link between the anticonvulsant and antioxidant efficacy of melatonin.

A tendency for increased expression in the frontal cortex and a significant upregulation in the hippocampus of HSP 70 was detected both in Wistar rats and SHRs after KA-induced SE. These results agree with previous findings that SE produced by systemic KA induced an expression of HSP 70 in neurons known to be susceptible to this neurotoxin and in the hippocampus, in particular (Gonzalez et al., 1989; Vass et al., 1989). Furthermore, a strong correlation between the duration of SE and the degree of HSP 70 expression was suggested by some authors (Lowenstein et al., 1990;

Shimosaka et al., 1992). Recently, several reports demonstrated increased sensitivity to heat-induced HSP 70 in vascular smooth muscle cultures, vibroblast cultures and other tissue in genetically hypertensive rodents (Hamet et al., 1990 A,B; Lukashev et al., 1991). In addition, an increased transcription of many HSP family genes, including HSP60, HSP70 and HSP90, and heat shock factor-1 have been reported in hypertensive rats (Zhou et al., 2005). In the present study we established that melatonin pretreatment over 14 days via s.c. implanted osmotic minipump prevented the KA-induced increased expression of HSP 70 in the hippocampus of Wistar rats. Curiously, melatonin failed to prevent the increased expression of HSP 70 in a model of essential hypertension. We can suggest that the low efficacy of melatonin is related to the fact that in SHRs the cellular homeostasis and defence antioxidant enzyme system is disturbed in naive SHRs compared to normotensive Wistar rats. The observed decreased LP level as well as the enhanced cytosolic and mitochondrial SOD, which are accompanied by a decreased expression of the HSP 70 in the hippocampus of Wistar rats pretreated with melatonin support the suggestion that there exist a close relationship between ROS and the expression of members of the Hsp70 family (Kukreja et al., 1994; Ambrosio et al., 1995; Lee J. Y. and Corry, 1998).

The concentration of melatonin is higher in brain ventricles than in the peripheral plasma following its exogenous administration (Tan et al. 2010). Because of its proximity to the ventricles, the hippocampus is one of the most peculiar brain structures, which may be susceptible to the action of melatonin (El Sherif et al. 2002). This may explain why in our study melatonin was more effective in the hippocampus than the prefrontal cortex as concern the changes in LP, cytosolic and mitohondrial SOD and HSP 70. According to these findings, the pattern of oxidative injury induced by KA seems to be highly region-specific.

In conclusion, SHRs showed increased seizure susceptibility and disturbed defense antioxidant system compared to normotensive Wistar rats. Subchronic systemic melatonin treatment exerted a mild anticonvulsant effect following KA injection in SHRs but not in Wistar rats. However, the efficacy of melatonin in preventing the KA-induced changes in the markers of oxidative stress and neurotoxicity was more pronounced in Wistar rats than in SHRs suggesting a lack of a direct link between the seizure activity and these markers.

Ascknolegements:

This work was supported by the Medical Science Council, Medical University of Pleven contract No. 11/2012 and National Science Fund contract No DTK 02/56, 2009–2012.

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Table 1

Effect of pretreatment with melatonin infused subcutaneously via osmotic minipumps for 14 days (10 mg/kg per day) on KA- induced seizures in Wistar and spontaneously hypertensive rats (SHRs). Analysis of data by two-way ANOVA indicated a main Strain effect [$F_{1,34=}$ 10.014, $p<0.003$] and Strain x Drug interaction [$F_{1,34=}$ 5.129, $p<0.031$] for the latency of KA-induced seizures. $^{o}P=0.02$ versus Wistar-KA-veh group; **P = 0.034 versus SHRs-KA-veh group (Kruskal-Wallis test)

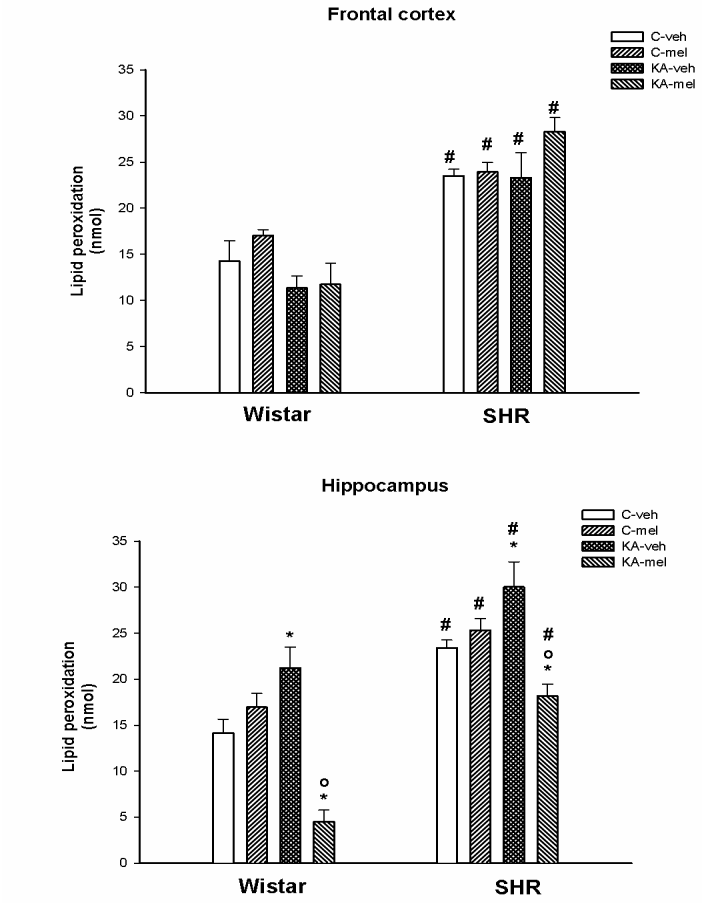

Fig. 1

Lipid peroxidation in the frontal cortex (A) and the hippocampus (B) of controls (C) or KA-treated Wistar and SHRs infused with vehicle (veh) or melatonin (mel) (details in the text to Table 1). Data are presented as means ± SEM (n = 10). Analysis of data by three-way ANOVA indicated a main Strain effect [$F_{1,63=}$ 69.337, p<0.001] in (A), a main Strain effect [$F_{1,63=}$ 66.905, p<0.001], a main Drug effect [$F_{1,63=}$23.638, p<0.001] and KA-treatment x Drug interaction [$F_{1,63=}$ 46.070, p<0.001] in (B). *p < 0.05 vs C-veh group; ^{o}p < 0.05 vs KA-veh group, $^{\#}$p < 0.05 vs Wistar rats.

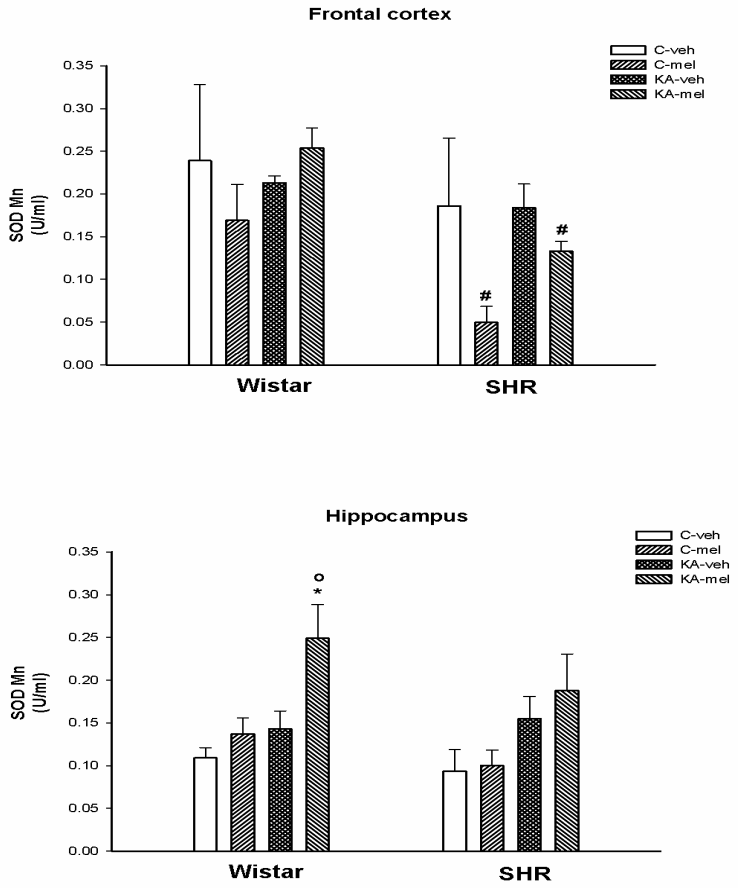

Fig. 2

Cytosolic superoxide dismutase (SOD Cu/Zn) activity in the frontal cortex (A) and the hippocampus (B) of controls (C) or KA-treated Wistar and SHRs pretreated with either vehicle (veh) or melatonin (mel) (details in Table 1). Data are presented as means ± SEM (n = 10). Analysis of data by three-way ANOVA indicated a main Strain effect [$F_{1,61=}$ 49.083, p<0.001], a main KA-treatment effect [$F_{1,61=}$ 42.620, p<0.001] and Strain x KA-treatment x Drug interaction [$F_{1,61=}$ 42.139, p<0.001] in (A). Analysis by three-way ANOVA showed a main KA-treatment effect [$F_{1,62=}$ 19.777, p<0.001], a main Drug effect [$F_{1,62=}$ 27.248, p<0.001], interaction Strain x KA-treatment x Drug [$F_{1,62=}$ 9.357, p=0.003] in (B). *p < 0.05 vs C-veh group; ^{o}p < 0.05 vs KA-veh group, $^{\#}$p < 0.05 vs Wistar rats.

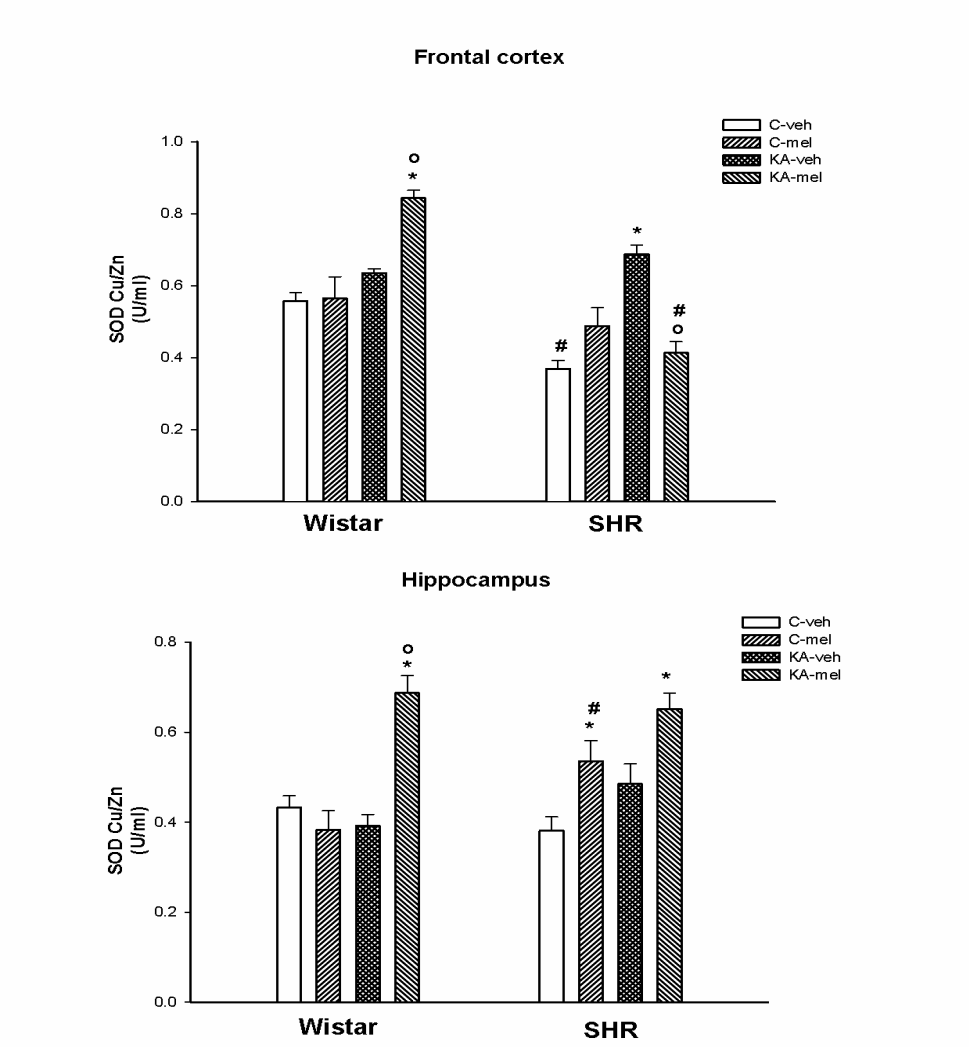

Fig. 3

Mitochondrial superoxide dismutase (SOD Mn) activity in the frontal cortex (A) and the hippocampus (B) of controls (C) or KA-treated Wistar and SHRs pretreated with either vehicle (veh) or melatonin (mel) (details in Table 1). Data are means ± SEM (n = 10). Analysis of data by three-way ANOVA indicated a main KA-treatment effect [$F_{1,68=}$ 14.472, $p<0.001$] in (B) and main Strain effect [$F_{1,62=}$ 7.979, $p=0.007$] in (A). *$p < 0.05$ vs C-veh group; $^{o}p < 0.05$ vs KA-veh group, $^{\#}p < 0.05$ vs Wistar rats.

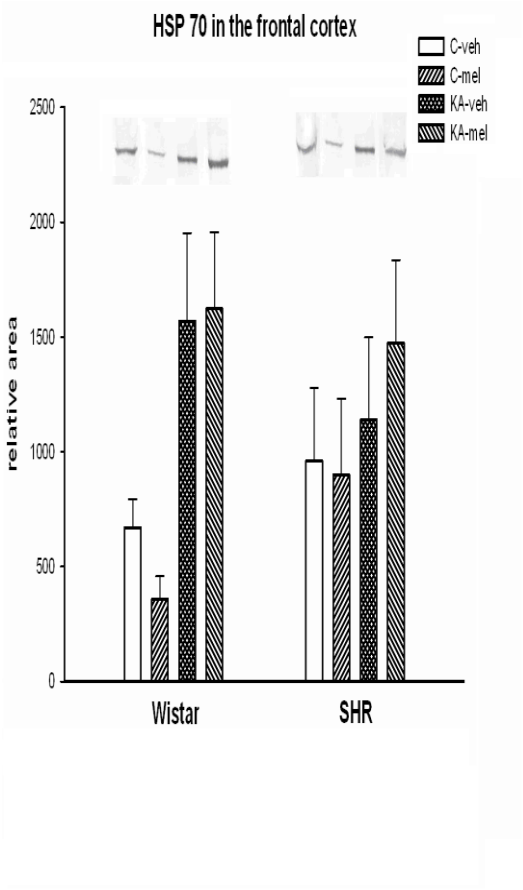

Fig. 4

Heat shock protein (HSP) 70 in the frontal cortex (A) and the hippocampus (B) of controls (C) or KA-treated Wistar and SHRs pretreated with either vehicle (veh) or melatonin (mel) (details in Table 1). Data are means ± SEM (n = 10). Analysis of data by three-way ANOVA indicated a main KA-treatment effect [$F_{1,55=}$ 9.308, p=0.004] in (A), a main Strain effect [$F_{1,52=}$ 18.725, p<0.001], a main KA-treatment effect [$F_{1,52=}$ 18.167, p<0.001] and interaction Strain x KA-treatment x Drug [$F_{1,52=}$5.088, p=0.029]. *p < 0.05 vs C-veh group; ^{o}p < 0.05 vs KA-veh group, $^{\#}$p < 0.05 vs Wistar rats.