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Agmatine reduces chlorpromazine prooxidant effects in rat hippocampus and striatum

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Abstract: The use of the antidepressant drug chlorpromazine (CPZ) is linked to the occurrence of oxidative stress in some brain structures. Thus, overcoming the side effects of CPZ is of great importance. Because agmatine (AGM) can act as a free radical scavenger, it is an interesting compound as an adjunct to CPZ therapy. The aim of our study was to investigate the enzymatic parameters of oxidative stress in the hippocampus and striatum of rats after CPZ treatment, and the potential protective effects of AGM. Rats were injected as follows with (i) 1 mL/kg b.w. saline; (ii) a single intraperitoneal (i.p.) dose of CPZ (38.7 mg/kg); (iii) CPZ (38.7 mg/kg) and AGM (75 mg/kg); (iv) AGM (75 mg/kg). CPZ induced an increase in superoxide anion radical ($O_2^{\bullet-}$) concentration, while the activities of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), were lowered in both the hippocampus and striatum. Cotreatment with CPZ and AGM protected the examined brain structures by reversing the antioxidant enzyme activities to the control values. Following CPZ treatment, the effects were more pronounced for SOD and GPx in the hippocampus, and for SOD, CAT and GPx in the striatum. The full effect of restored superoxide production was achieved in the striatum, which points to the role of CAT. The obtained results suggest that CPZ in combination with AGM may be considered as a new treatment strategy.

Keywords: chlorpromazine; agmatine; oxidative stress; hippocampus; striatum

INTRODUCTION

The use of antipsychotic drugs for the treatment of psychiatric syndromes such as schizophrenia, which affect almost 1% of the world population [1], is routinely applied. Chlorpromazine (CPZ) is an antipsychotic drug widely used for treating schizophrenia, bipolar disorder, severe anxiety, psychotic aggression and other disorders [2,3]. CPZ affects the mesolimbic pathway through a postsynaptic blockade of dopaminergic receptors, altering the turnover and release of dopamine (DA) [4,5]. Consequently, an increase in DA levels in different brain regions such as the hippocampus and striatum, was observed after CPZ administration [6]. Blockage of D2 receptors in the nigrostriatal pathway is responsible for its extrapyramidal side effects, which could be linked to increased body weight, higher risk for diabetes, hypertension and cardiovascular disease [6-8]. Also, antipsychotic drugs were shown to affect the oxidative status in different organs [9,10]. Classic antipsychotics raise oxidative stress by altering the levels of antioxidant enzymes and cause oxidative injury in the brain [11]. Increased lipid peroxidation and activities of glutathione reductase (GR), glutathione peroxidase (GPx), and decreased activities of catalase (CAT) and superoxide dismutase (SOD) after CPZ administration were reported in African catfish brain [12]. Also, variations in the activities of antioxidant enzymes were described

in plasma, red blood cells and the cerebrospinal fluid of patients treated with the antipsychotics [13].

Agmatine (AGM) is a polycationic amine recognized as a bioregulator of different cell functions. AGM is synthesized from L-arginine by arginine decarboxylase [14-16]. It is also known for treating a various range of pathologies, such as diabetes, insulin resistance, neuropathic pain, traumatic brain injury, depression, etc. [17]. AGM is also recognized as a free radical scavenger in its ability to decrease lipid peroxidation, increase SOD activity and/or reduce the glutathione content [18,19]. Considering the long-term safety of high daily doses of dietary AGM [20], it could be considered as a novel therapeutic agent for different pathologies, including those pathologies provoked by drugs.

The aim of the present study was to investigate the potential ability of AGM to diminish the prooxidant side effects observed after CPZ administration. We hypothesized that a combined treatment with CPZ+AGM can decrease the prooxidative effect of CPZ, with a concomitant increase in antioxidative enzyme activities in the hippocampus and striatum of experimental male rats.

MATERIALS AND METHODS

Experimental animals

Animal experiments were approved according to governmental regulations (Official Gazette RS, No. 14/2009) and EU Directive 2010/63/EU, supported by the Ethics Committee of the Military Medical Academy and by the Veterinary Department of the Ministry of Agriculture and Environmental Protection of the Republic of Serbia (License No. 323-07-03937/2016-05/7). The experiments were performed on adult, 2-month-old male Wistar rats weighing about 230 g. The animals were housed under standard environmental conditions (a 13-h light/11-h dark cycle, 23±2˚C, 55±10% humidity) and fed with a standard chow diet for laboratory rats and tap water *ad libitum*.

Experimental procedure

Rats were randomly placed into four groups (n=10), as follows: the control (C) group was injected with saline (1 mL/kg b.w.); the CPZ group received a single dose of chlorpromazine-HCl (38.7 mg/kg b.w.; Medisca, Italy); the CPZ+AGM group was injected with 38.7 mg/kg b.w. CPZ-HCl, followed by the administration of 75 mg/kg b.w. AGM; the AGM group received 75 mg/kg b.w. of AGM; all animals were injected intraperitoneally (i.p.) once.

Tissue preparation

Fourty-eight h after injection, the animals were anesthetized, perfused with 0.9% saline for 5 min and killed by decapitation. The brain was removed, and the striatum and hippocampus were isolated. The procedure of tissue preparation was performed on ice. About 100 mg of the striatum and hippocampus were transferred into 1 mL of ice-cold sucrose (0.25 mol/L sucrose, 0.1 mmol/L EDTA) in sodium-potassium phosphate buffer, pH 7.2, and placed into a glass tube homogenizer (Tehnica Zelezniki Manufacturing, Slovenia). Homogenization was performed two time with a Teflon pestle at 800 rpm for 15 min at 4°C. The homogenates were centrifuged at 2500 x g for 30 min, at 4°C. The supernatants were sonicated in three cycles (30 s sonication, 15 s pause) at 10 kHz. The protein content was quantified according to the method described by Lowry et al. [21], using bovine serum albumin as standard.

Determination of superoxide anion radical ($O_2^{\bullet-}$) concentration

The $O_2^{\bullet-}$ content was quantified by the method based on the reduction of nitroblue-tetrazolium (NBT Sigma-Aldrich, Munich, Germany) to monoformazan by $O_2^{\bullet-}$ in an alkaline nitrogen saturated medium, which is expected to decrease the oxygen tension in the medium. The yellow color of the reduced product was measured spectrophotometrically at 550 nm [22]. The concentration of $O_2^{\bullet-}$ was expressed as μmol of reduced NBT/mg protein.

Determination of total superoxide dismutase (tSOD) activity

The activity of tSOD was measured spectrophotometrically as the inhibition of the spontaneous autooxidation of epinephrine at 480 nm in a carbonate buffer

(50 mM, pH 10.2, containing 0.1 mM EDTA; Serva, Feinbiochemica, Heidelberg, Germany), after the addition of 10 mM of epinephrine (Sigma, St. Louis, MO, USA) [23]. The results were expressed as units of SOD per mg proteins, with one unit of SOD ativity defined as the activity of the enzyme that caused 50% inhibition of epinephrine.

Determination of CAT activity

CAT activity was determined spectrophotometrically by monitoring the formation of a yellow complex of ammonium molybdate (Serva, Feinbiochemica, Heidelberg, Germany) with H_2O_2 [24]. Kinetic analysis was performed at 405 nm. Data were expressed as mU of CAT per mg of protein. One unit of CAT activity is defined as μM H_2O_2/min/mg protein.

Determination of GPX activity

The method is based on a spectrophotometric measurement of NADPH consumption at 340 nm. GPx catalyzes the reduction of (lipid) hydroperoxides to alcohols using reducing equivalents of GSH, which subsequently becomes oxidized. Regeneration of the depleted GSH occurs throughout the reduction of GSSG to GSH, catalyzed by GR, which utilizes NADPH as a donor of the reducing equivalents. The reduction of every mole of GSSG requires one mole of NADPH [25]. Results were expressed as U of GPx per mg of protein. One unit of GPx activity is defined as nM NADPH/min/mg protein.

Determination of GR activity

Glutathione activity was determined spectrophotometrically [26]. The method is based on the ability of GR to catalyze the reduction of GSSG to GSH by the oxidation of NADPH to $NADP^+$. Nicotinamide adenine dinucleotide (NAD, 100 mmol) was used as standard. One unit of GR activity is described as μmol NADPH/min/mg proteins. The results were expressed as mU per mg of proteins.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 SoftwareR (GraphPad Software, La Jolla, CA, U.S.). Kruskal-Wallis one-way analysis of variance (ANOVA) followed by Dunn's *post hoc* test was performed. The data were presented as the mean±standard deviation and considered statistically significant for $P<0.05$.

RESULTS

CPZ treatment induced a significant increase ($P<0.001$, 67%) in $O_2^{\bullet-}$ production in rat hippocampus when compared to the control group. Cotreatment with CPZ and AGM induced a decrease ($P<0.001$, 9%) in $O_2^{\bullet-}$ in comparison to CPZ treatment in the hippocampus. A significant increase ($P<0.01$, 44%) in $O_2^{\bullet-}$ after CPZ treatment in the striatum was detected in comparison to the control value. Cotreatment with CPZ and AGM decreased ($P<0.05$, 21%) $O_2^{\bullet-}$ in comparison to the CPZ treatment. The AGM treatment led to a significant decrease ($P<0.05$) in $O_2^{\bullet-}$ concentration in both structures in comparison to the CPZ group. (Fig. 1).

After treatment with CPZ there were no significant changes in CAT and GR activities in the hippocampus compared to the control values. The activities of tSOD and GPx in the hippocampus after CPZ administration were significantly decreased ($P<0.05$ and $P<0.001$, by 23% and 48%, respectively) in comparison to the control group. The combined treatment with CPZ and AGM increased antioxidant enzyme activities. AGM treatment caused significant increases in tSOD and GPx activities ($P<0.05$; $P<0.001$, respectively) in the hippocampus in comparison to the CPZ group (Fig. 2). In the striatum after CPZ treatment CAT, tSOD and GPx activities were significantly decreased in comparison to the controls ($P<0.05$, $P<0.01$ and $P<0.001$; 22%, 27%, and 50%, respectively). Cotreatment with CPZ and AGM reversed the examined parameters to the control levels. No significant differences were observed between the control and AGM group (Fig. 3).

DISCUSSION

Treatment with antipsychotic drugs is challenging due to their adverse effects. Oxidative stress is one of the negative effects observed after CPZ treatment, be-

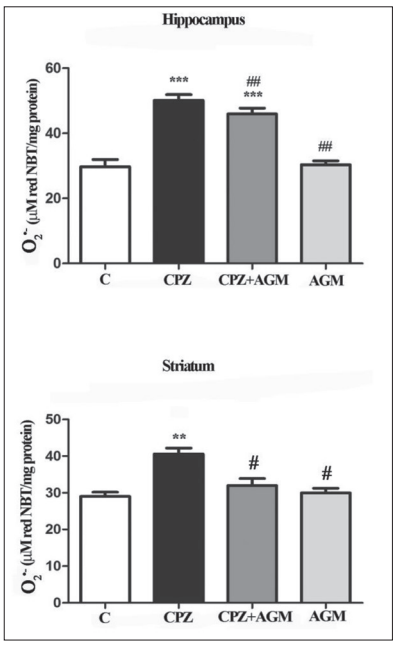

Fig. 1. Superoxide anion radical ($O_2^{\bullet-}$) content in rat hippocampus and striatum from control (C), chlorpromazine (CPZ), chlorpromazine and agmatine (CPZ+AGM) and agmatine (AGM) groups 48 h after treatment. Levels of significance: * – compared to the control group (C); # – compared to chlorpromazine-group (CPZ). The data are presented as the means±SD and were considered statistically significant for P<0.05.

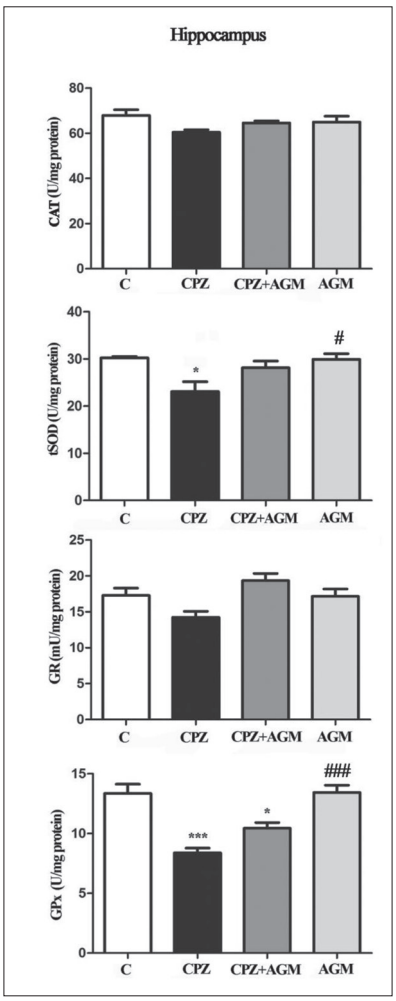

Fig. 2. Total superoxide dismutase (tSOD, U/mg protein), catalase (CAT, U/mg protein), glutathione reductase (GR, mU/mg protein) and glutathione peroxidase (GPx, U/mg protein) activities in rat hippocampus from control (C), chlorpromazine (CPZ), chlorpromazine and agmatine (CPZ+AGM) and agmatine (AGM) groups 48 h after treatment. Significance compared to the * – control group (C), # – chlorpromazine-group (CPZ). Data are presented as the means±SD and were considered statistically significant for P<0.05.

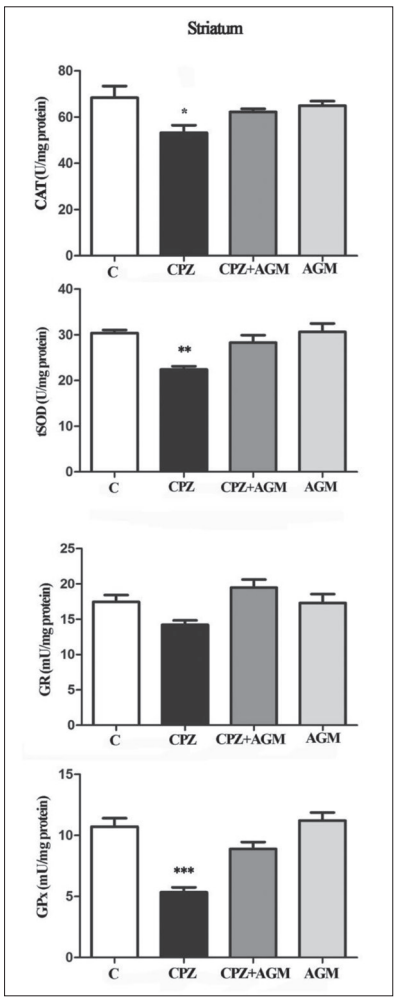

Fig. 3. Superoxide dismutase (SOD, U/mg protein), catalase (CAT, U/mg protein), glutathione reductase (GR, mU/mg protein) and glutathione peroxidase (GPx, U/mg protein) activities in rat striatum from control (C), chlorpromazine (CPZ), chlorpromazine and agmatine (CPZ+AGM) and agmatine (AGM) groups 48 h after treatment. Significance compared to the * – control group (C), # – chlorpromazine-group (CPZ). Data are presented as the means±SD and were considered statistically significant for P<0.05.

cause of its prooxidant properties [27, 28]. Also, the brain is very vulnerable to oxidative stress due to its higher consumption of oxygen and subsequent generation of reactive oxygen species (ROS). AGM is recognized as a free radical scavenger because of its antioxidant effect in the brain and cytoprotective effect [17]. We examined whether AGM has the potential to reduce the adverse effects of CPZ application in rat hippocampus and striatum. The parameters of oxidative stress and antioxidant capacity were altered after CPZ treatment. Increased $O_2^{\bullet-}$ production pointed to increased oxidative stress in the hippocampus and striatum. Previous studies revealed a potent prooxidative effect of CPZ [28,29,30], as well as decreased activities of MnSOD and CuZnSOD, decreased GSH and increased lipid peroxidation levels [31,32]. CPZ has the potential to be oxidized into the CPZ cation radical, which is highly reactive in the presence of H_2O_2 or other free radicals [33]. Overproduction of $O_2^{\bullet-}$ may be a consequence of dopamine autooxidation. Dopamine metabolism is followed by the

formation of H_2O_2, which reacts with Fe or Cu ions to produce the highly reactive hydroxyl radicals (•OH). Neuroleptics can block dopaminergic receptors and increase dopamine turnover [4, 5], and enhance H_2O_2 generation, resulting in oxidative stress. Increased $O_2^{\bullet-}$ production in the hippocampus and striatum after 48 h of treatment highlighted the long-term toxic effect of CPZ caused by disturbance of the redox balance. Increased $O_2^{\bullet-}$ production is accompanied by decreased GPx activity. Increased $O_2^{\bullet-}$ production was also accompanied by decreased SOD activity in the striatum after 48 h of CPZ administration, indicating that the activity of this enzyme was insufficient to eliminate it, and lead to oxidative stress. Also, our previous studies revealed increased nitrosative stress in the hippocampus and striatum after acute CPZ treatment [33]. Decreased CAT activity in the striatum after 48 h of CPZ administration may be linked to lower activity of SOD, with superoxide converted to hydrogen peroxide, which is the substrate for CAT. Previous results showed reduced GSH concentration in the cortex, striatum and hippocampus, which indicates antioxidant system impairment [34]. The role of the GSH in protecting cells from toxic effects is reflected in its antioxidant effect, as it neutralizes ROS within the cell via the GPx/GSH cycle [35]. The rate of inactivation of SOD is directly dependent on the concentration of H_2O_2 and the enzyme itself [36]. The combined treatment of CPZ and AGM reduced oxidative stress in both brain structures. In the present study, we demonstrated that an acute CPZ and AGM administration produced significant $O_2^{\bullet-}$ production in the hippocampus and reversed the activity of GPx in both the hippocampus and striatum after 48 h of treatment as compared to the treatment with CPZ only. The reversed activity of GPx 48 h after AGM+CPZ administration in rat hippocampus is the result of increased H_2O_2 production. CAT and GSH-Px are the main antioxidant components involved in H_2O_2 elimination. The Michaelis-Menten constant (Km) for H_2O_2 in CAT is significantly higher than in GSH-Px [37]. Thus, GSH-Px is efficient in H_2O_2 elimination when its concentration is near the physiological level [38], and CAT is efficient during H_2O_2 overproduction. Combined application of AGM and CPZ reverses the activities of antioxidant enzymes and reduces superoxide anion production, thereby preventing oxidative stress. The favorable effect of AGM could be related to its blocking of ion channels, suppression of damaging ROS, blocking of N-methyl-D-aspartate (NMDA) receptors, promotion of neurogenesis, angiogenesis [39]. Furthermore, AGM inhibits nitric oxide synthase NOS mRNA expression and stops the increase of oxidative stress [40]. It also inhibits the production of cytokines and inflammation [41] and possesses powerful inhibitory activity in normal and disease conditions [40].

In conclusion, the study shows that the negative effects of CPZ treatment were more pronounced for tSOD and GPx in the hippocampus and for tSOD, CAT and GPx in the striatum. The full effect of restored superoxide production was achieved in the striatum, which points to the role of CAT. The obtained results suggest that CPZ in combination with AGM can be considered as a treatment strategy in pathological states when the use of the antipsychotic CPZ is warranted.

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Author contributions: Svetlana Trifunović and Ivana Stevanović: manuscript concept creators who wrote the manuscript and interpreted the results in a broader context of the available literature; Bratislav Dejanović and Milica Ninković organized and conducted the experiments (work in the animal unit, care and treatment, brain extraction, etc.; Vesna Begović-Kuprešanin processed the experimental material; Irena Lavrnja and Branka Šošić-Jurjević performed some measurements, designed the figures, and discussed aspects of the results.

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