

EFFECT OF ANTHOCYANINS AND MIANSERIN ON NEURONAL DENSITY IN RAT HIPPOCAMPUS IN A MODEL OF OXIDATIVE STRESS

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Summary

Oxidative stress is associated with depressive disorder and selective loss of hippocampal volume. We have previously demonstrated that anthocyanins showed antidepressive activity comparable to this of Mianserin in an experimental model of chronic oxidative stress. The aim of the present study was to investigate the effect of combined application of anthocyanins and Mianserin on stress-related neuronal damages in CA3 hippocampal area of rat brain.

Male Wistar rats were separated in four groups: I group – control; II group - rats, exposed to constant light for a period of 14 days; III group – rats, exposed to constant light for a period of 14 days and treated with anthocyanins during the light stress; on the 15th day Mianserin was added for a period of 7 days; IV group – after 14 days of light stress rats were treated with Mianserin for 7 days. The neuronal density in the hippocampal subregion was determined by Nissl staining.

Combined application of anthocyanins and Mianserin significantly increased neuronal density in CA3 hippocampal region of rats, exposed to chronic stress caused by constant light exposure.

Our results suggest that anthocyanins may play beneficial role in the therapy of mental disorders.

Key words: anthocyanins, mianserin, depression, oxidative stress, rat, hippocampus

Introduction

The great prevalence of depressive disorders, the inadequate present therapy and the growing number of drug-resistant forms of Major Depressive Disorder (MDD) determined the researchers' interest to this most common mental disorder.

Depression is determined as stress-related disorder [1, 2]. Oxidative stress plays a role not only in the development of depression but in the neuronal loss [3]. An important structure associated with the pathophysiology of MDD is the hippocampus [4, 5]. Experimental and clinical studies demonstrate decreased volume and atrophy of the hippocampus in a state of depression [6, 7, 8]. This is observed particularly in the CA3 hippocampal region, which is related to neurogenesis [9, 10].

Furthermore, deficits in memory functions and impairment of cognitive processes are very common among MDD patients [11, 12]. Antidepressants can correct these changes to a great extent, however their effect sets in slowly. So, the question is whether the effective treatment at an early stage of depressive disorder can hold up or prevent the changes in rat hippocampus.

Anthocyanins are known as powerful natural antioxidants. Evidence suggests that diurnal rhythm disruption, due to chronic exposure to constant light caused oxidative stress in rat brain, liver and kidney tissues [13]. We have previously shown that the exposure of male rats to constant light led to development of depressive-like symptoms [14]. We also demonstrated that anthocyanins decreased oxidative stress and depressive symptoms with or without the antidepressant drug Mianserin [15]. Mianserin predominantly stimulates the noradrenergic neurotransmission by inhibition of α_2 -presynaptic receptors. Some investigations indicate that anthocyanins protect catecholamines from oxidation and support their neurotransmission [16]. Thus, there was a reason to expect that the effect of the antidepressant would be more prominent when combined with anthocyanins.

The effect of combined application of anthocyanins and antidepressants on hippocampal neuronal density is not documented. The aim of our study was to determine whether the early administration of antioxidants would protect rat brain from oxidative damages and would enhance the effect of the antidepressant. We evaluated the effect of the simultaneous application of anthocyanins and Mianserin on CA3 hippocampal neuronal density of rats, exposed to diurnal rhythm disruption, caused by exposure to chronic constant light.

Materials and Methods

Experimental animals

Male Wistar rats (270-330 g) were housed in groups of five with free access to food and water and maintained in a temperature and humidity controlled room. All animals were handled in the laboratory room for 5 minutes once a day 2-3 days prior to procedures. The experimental rats were separated in 4 groups (n = 5) and treated as follows:

I group – control, with normal diurnal rhythm and treated p.o. with vehicle for a period of 14 days.

II group – “stress” – rats were exposed to constant light and treated p.o. with vehicle for a period of 14 days

III group – “stress + Mianserin” – rats were exposed to constant light and treated p.o. with vehicle for a period of 14 days; on the 15th day Mianserin was administered for 7 days p.o., in a dose of 30 mg/kg.

IV group – “stress + Mianserin + anthocyanins” - rats were exposed to constant light and treated with water solution of anthocyanins p.o. in a dose of 200 mg/kg for a period of 14 days; on the 15th day Mianserin was administered p.o. in a dose of 30 mg/kg in combination with anthocyanins for 7 days.

Each substance was given per os in a volume of 0,2 ml/100 g body weight, at a 10-min interval.

The experiments were carried out in accordance with the Bulgarian regulations on animal welfare and in conformance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Substances

Anthocyanins, total dry standardized concentrate, extracted from red wine (Dr. Winfred Berr, Germany), soluble in water.

Mianserin HCl (Pharmacia AD, Bulgaria).

Methods

- Model of oxidative stress – a modified model by Baydas et al. (2001) [13]. The experimental animals were exposed to constant light for 14 days.
- Light microscopy. Paraffin-embedded and Nissl-stained preparations were used for studying the neuronal density. The animals were deeply anesthetized with Thiopental (40 mg/kg) and fixed by transcardial perfusion with 10 % formaldehyde. The brains were cut transversally at 10- μ m-thick sections and stained with cresyl violet. Morphometric analysis was performed using the microanalysis system Olympus CUE-2 (primary magnifications 20 \times and 40 \times). Data were entered in the computer program, recorded automatically and calculated.
- Data analysis. The results were expressed as mean number of neurons per $\text{mm}^2 \pm$ STDEV. Statistical analysis was performed using one-way ANOVA, followed by Dunnett's test; $p < 0,05$ was considered statistically significant.

Results

Figure 1 shows the effects of Mianserin and the combination of Mianserin and anthocyanins on the neuronal density in CA3 hippocampal area of rats, exposed to constant light.

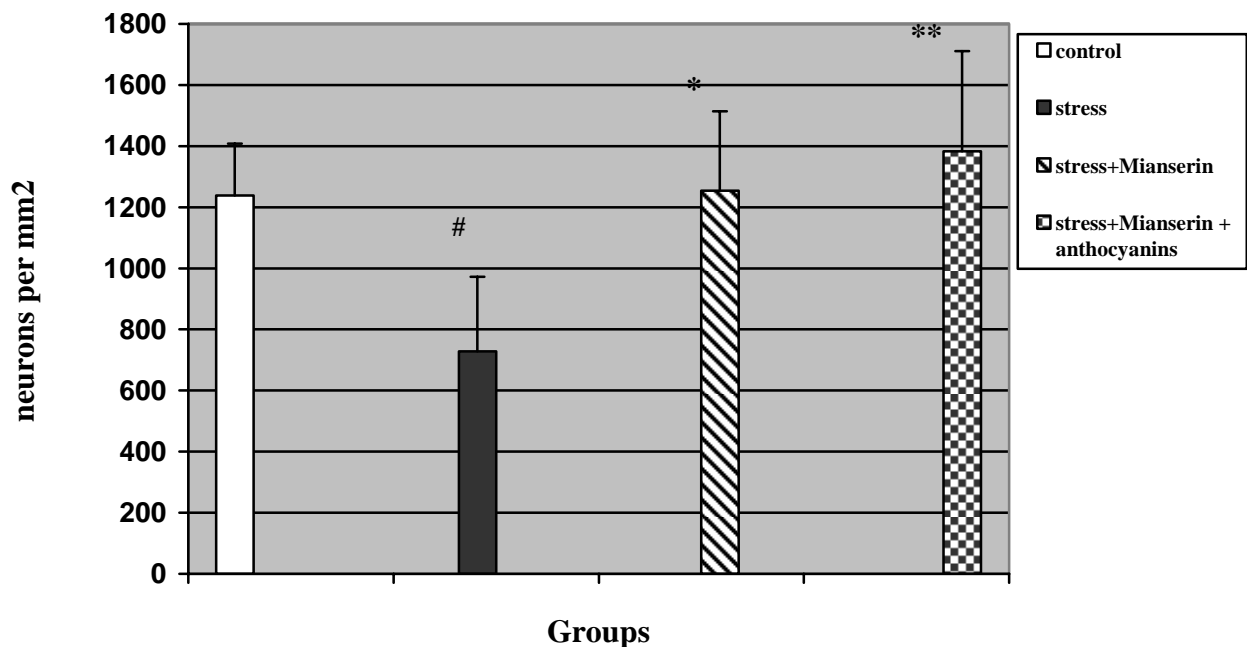


Fig. 1. Number of Nissl stained neurons per mm^2 in CA3 hippocampal area ($n = 5$).
$p < 0,05$ vs the control group; * $p < 0,05$ and ** $p < 0,01$ vs the “stress” group.

The results showed that the light stress significantly decreased the neuronal density in comparison to the control group ($p < 0,05$). Treatment with Mianserin significantly increased the neuronal density compared to the “stress” group ($p < 0,05$). The combined application of Mianserin and anthocyanins exceeded the effect of Mianserin and led to more significant enhancement of the neuronal density ($p < 0,01$). The neuronal density in the III and IV group was comparable with the control, non-stressed group.

Discussion

Our results demonstrated that chronic stress developed via diurnal rhythm disruption decreased the neuronal density in CA3 hippocampal region of male rats. Treatment with Mianserin significantly enhanced neuronal density. The application of anthocyanins in the course of the oxidative stress protected brain neurons against free radicals and diminished neuronal loss. The combined application of anthocyanins and Mianserin exceeded the effect of Mianserin on neuronal density in CA3 hippocampal region.

In a state of oxidative stress high free radical levels lead to excitotoxicity, activate apoptosis, and decrease neurogenesis [5, 17]. Mc Ewen et al. (2001) demonstrated that repeated stress caused atrophy on dendrites in the CA3 region [10]; both acute and chronic stress suppressed neurogenesis of dentate gyrus granule neurons [11, 12]. Extensive investigations have shown that prolonged stress has adverse effects on rodent hippocampus and hippocampal changes are associated with memory deficits [10, 18].

Our results showed decreased neuronal density in CA3 hippocampal region of rats during chronic light stress. Moreover, our previous studies demonstrated depressive-like symptoms and cognitive deficit in male rats, exposed to constant light for a period of 14 days [14]. Our data suggest that depressive symptoms and cognitive deficit might be related to neuronal loss in hippocampus of rats, exposed to oxidative stress.

It is established that antidepressants stimulate the expression of neurotrophic factors, increase the density, the length and the arborization of the dendrites [19] and enhance the synaptic plasticity [9, 19, 20]. Literature data show that antidepressant treatment up-regulates hippocampal neurogenesis and is able to block or reverse the atrophy and damages caused by stress [21]. However, the conventional antidepressants require a lot of time to display their therapeutic effectiveness.

Our results demonstrate that the antidepressant Mianserin increased CA3 neuronal density in rats, exposed to stress, due to diurnal rhythm disruption. There are controversial data about the effect of various antidepressants on hippocampal neuronal loss. Luo L. and Tan R. (2001) reported that the selective 5-HT reuptake inhibitor fluoxetine inhibited dendrite atrophy of hippocampal neurons in a model of chronic mild stress [22]. On the other hand, Magarinos et al. (1999) found that fluoxetine and fluvoxamine failed to block dendritic atrophy in CA3 pyramidal neurons after chronic stress. In contrast, the 5-HT reuptake enhancer (+/-) tianeptine prevented the dendritic atrophy caused by repeated restrained stress [19]. The studies on Mianserin suggest that both noradrenergic and serotonergic neurotransmission may play roles in hippocampal function. Further studies will determine the involvement of both neurotransmissions in control of neuronal density of rats, exposed to constant light.

Anthocyanins are known as plant antioxidants with variety of biological activities and therapeutic benefits [23]. They possess neuroprotective effects [24]. Our previous studies showed that anthocyanins in a dose of 200 mg/kg displayed antidepressive-like activity [25]. The present results demonstrate that anthocyanins protected the hippocampal neurons during chronic stress. In addition, the application of anthocyanins enhanced the beneficial effect of Mianserin on CA3 hippocampal neurons. We suppose that the reported protective effect of anthocyanins on brain neurons is related not only to their antioxidant activity. We suggest that the neuroprotective properties of anthocyanins might be due to influence on different enzyme systems, transcriptional factors [26], inhibition of PDE, increased cAMP levels [27, 28] and support of catecholamine neurotransmission. Taken together these data may explain the more prominent effect of the combined application of Mianserin and anthocyanins on CA3 neuronal density.

Our results suggest that anthocyanins as antioxidants and bioactive substances may play beneficial role in the therapy of mental disorders.

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