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ORIGINAL ARTICLE

Naltrexone improves immune functions and spatial memory of ovariectomized rats with alcohol intake

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ABSTRACT

Estrogen deficiency and excessive alcohol intake are associated with numerous adverse effects on immune functions and cognitive abilities. There is lots of evidence for the role of cytokines in the functions of the central nervous system like emotions, learning and memory. The aim of the present study was to investigate the effects of the opioid antagonist naltrexone on IFN-γ serum levels and spatial memory of ovariectomized (OVX) rats with repeated alcohol intake. Female Wistar rats (200–200 g) were ovariectomized and randomly divided in four groups: 1 – "control" (physiological solution 1 ml/100 g p.o. for 4 weeks after OVX); 2 – "alcohol" (ethanol 30%, 1 ml/100 g, p.o. for 4 weeks after OVX); 3 – "alcohol+naltrexone" (ethanol 30%, 1 ml/100 g, p.o. for 4 weeks after OVX and naltrexone 2.5 mg/kg, i.p. for 3 weeks after OVX); 4 – "naltrexone" (naltrexone 2.5 mg/kg, i.p. for 3 weeks after OVX). Our results suggested a correlation between IFN-γ levels and learning and memory abilities of the experimental rats. Moreover, we demonstrated that naltrexone reduced the adverse effects of OVX and excessive ethanol on IFN-γ levels and cognitive performance. Our data suggest that naltrexone may have beneficial therapeutic effects on immunodeficiency and emotional and intellectual deficits in women with estrogen insufficiency and excessive alcohol intake.

KEY WORDS: ovariectomy; estrogen deficiency; alcohol; naltrexone; IFN-γ; spatial memory

Introduction

Estrogen deficiency is associated with a myriad of physical and psychological challenges, dysregulation of metabolic and immune functions, cognitive decline and emotional difficulties. Literature data show that menopause is often correlated with alcohol abuse (Epstein *et al.,* 2007; Taki *et al.,* 2014). Estrogen deficiency and excessive alcohol intake can both have deleterious effects on immune functions (Wang *et al.,* 1994; Kale *et al.,* 2014). Our previous experience (Boyadjieva & Sarkar, 2010), consistent with other experimental results (Jeong *et al.,* 2008; Franchi *et al.,* 2010), have shown that chronic alcohol consumption decreases the production of various cytokines. Moreover, we have demonstrated that the administration of the opioid receptor antagonist naltrexone increased the production of IFN-gamma in the splenocytes of pair-fed and alcohol-fed rats (Boyadjieva & Sarkar, 2010).

Estrogens play a neuroprotective role and are known to significantly modulate adult neurogenesis (Barker & Galea, 2008; Li *et al.,* 2011). Substantial evidence demonstrates that estrogen deficiency, caused by ovariectomy (OVX), impairs cognitive functions and induces memory deficits (Heikkinen *et al.,* 2004; Su *et al.,* 2012; Kim *et al.,* 2015). We have previously reported that experimental OVX disrupted cognitive parameters of female rats and developed depressive-like symptoms (Varadinova *et al.,* 2009). Also, it is well established that continuous alcohol consumption causes moderate to severe learning and memory dysfunctions. Compelling data from experimental models of chronic alcoholism demonstrate that the animals had impaired cognitive functioning and significant loss of performance in the radial maze tests (Gal & Bardos, 1994; Vetreno *et al.,* 2011).

Thus, considering: 1 – estrogen deficiency and its relationship with immune and cognitive functions; 2 – prolonged alcohol consumption and its consequences on cytokine production and learning and memory abilities; 3 – the correlation between estrogen deficiency and

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alcohol abuse; 4 – the effects of naltrexone on immunity and cognition and 5 – the absence of studies that investigate the influence of naltrexone in models of estrogen deficiency and chronic alcohol intake, the present study aims to investigate the effects of repeated naltrexone application on the plasma levels of IFN-gamma and spatial learning and memory functions in 8-arm radial maze of OVX rats with alcohol intake.

Materials and methods

Chemicals

The chemicals used in the investigation were from Sigma-Aldrich, Vienna, Austria and of finest grade. The 30% ethanol solution was prepared using ethyl alcohol ≥99.8%.

Animals

Female Wistar rats (220–260 g) were randomly assigned into four groups (n=10). The experimental animals were maintained under standard laboratory conditions (12 h light–dark cycle, temperature 22±1 °C, humidity 65±1%). Rats had free access to food and water, and were handled for 3–5 days prior to procedures.

Ovariectomy

The rats were allowed one-week acclimatization period before surgery. Ovariectomy was performed under Ketaminol (0,08 ml/100 g, i.p.) anesthesia. The surgical procedure involved bilateral removal of rat's ovaries. Complete ovariectomy was evaluated by visual inspection of the ovaries after their extraction. Each animal was housed individually and kept under close observation for approximately 2–4 h until full recovery from the anesthesia. Following the recovery period (approximately 24 h after surgery), the animals were grouped together. According to our experimental protocol the treatment with the substances started one week after the OVX.

I group – "control" (C) – the OVX rats received physiological solution 1 ml/100 g, p.o. for a period of 4 weeks after ovariectomy. **II group** – "alcohol" (A) – the OVX rats received ethanol 30%, 1 ml/100 g, p.o. for a period of 4 weeks after ovariectomy. **III group** – "alcohol+naltrexone" A+N) – the OVX rats received ethanol 30%, 1 ml/100 g, p.o. for a period of 4 weeks after ovariectomy and naltrexone 2.5 mg/kg, i.p. for a period of 3 weeks after ovariectomy. **IV group** – "naltrexone" (N) – the OVX rats received naltrexone 2.5 mg/kg, i.p. for a period of 3 weeks after ovariectomy.

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) and with the approval of Medical University-Sofia ethics committee.

Determination of IFN-γ levels

At the end of the second and at the end of the fourth week of alcohol treatment blood was taken from all animals for determination of IFN-γ levels. The quantitative determination of IFN-γ concentrations in picograms per milliliter was according to the protocol of RAT IFN-γ ELISA kit (cat. № BMS621 rIFN-γ, Bender MedSystems GmbH, Vienna, Austria).

8-arm radial maze test

8-arm radial maze test is used to assess the spatial memory of experimental animals (Olton, 1977). All procedures were carried out before feeding the animals. The radial maze had 8 arms, which were Plexiglas-opaque. All arms had two ends, one-ending in the middle circle. First, during the "adaptation", 3 rats of each group are placed in the radial maze, with each corridor already baited (except for one "free" corridor) and are left there for 10 minutes. The "adaptation" is carried out for 3 days. After that, each animal is placed alone in the labyrinth for 6 minutes every day. At this stage of "training" only 4 corridors are baited and their order is kept the same throughout the experiment. We observed the number of entries in the different corridors. Entering in a non-baited corridor is considered a Total error (TE). Entering the "free" corridor is a Reference memory error (RME) – a type of long-term memory. The re-entering in a corridor where the bait has already been eaten is a Working memory error (WME) – a type of short-term memory.

The tests in the 8-arm radial maze were carried out after the second and the fourth week of the alcohol intake (respectively after the first and the third week of the naltrexone treatment).

Data analysis

The data were analyzed with General Linear Model in the SPSS 11.0 computer program one-way ANOVA test. The results were expressed as mean levels of IFN-γ ± STDEV, mean seconds ± STDEV for latency time, and mean number of errors ± STDEV. A p-value <0.05 was considered statistically significant.

Results

The serum levels of IFN-γ were considerably decreased in the "alcohol" group (p<0.0001) and the "alcohol+naltrexone" group (p<0.001) compared to the control one (Figure 1). There were no significant differences in the IFN-γ levels between the "naltrexone" group and the control group.

There was statistically significant increase in the IFN-γ levels in the serum of the "alcohol+naltrexone" treated rats compared to the "alcohol" group (p<0.02). The rats receiving naltrexone alone showed considerably higher IFN-γ levels compared to the "alcohol" group (p<0.0001).

Alcohol intake for 2 weeks in the "alcohol" group significantly increased the number of TE (p<0.0001), WME (p<0.0001) and RME (p<0.001) vs the control group. The group treated with naltrexone for 1 week and receiving alcohol for 2 weeks demonstrated considerably higher number of errors vs the control group – TE (p<0.01), WME (p<0.0001) and RME (p<0.01). Also, these rats had

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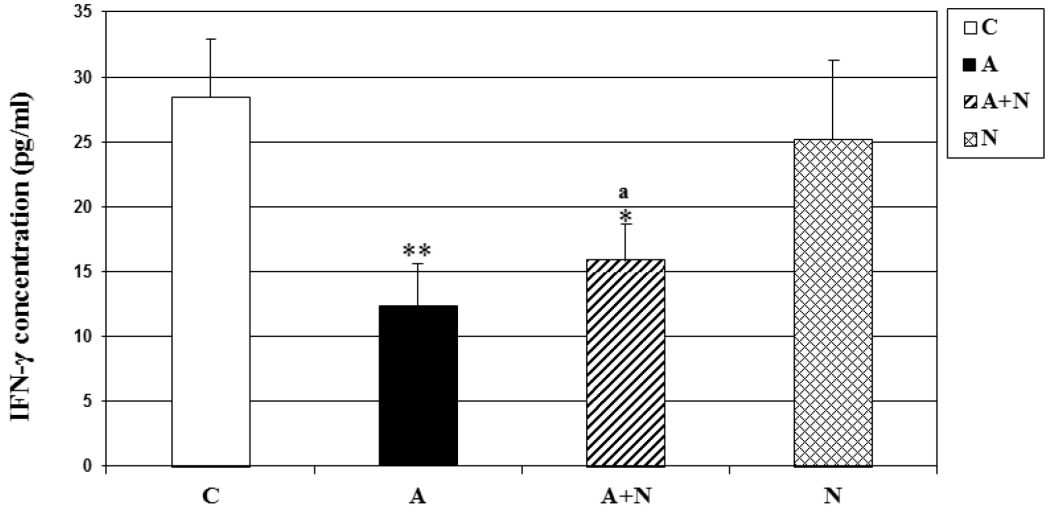

Figure 1. Effect of alcohol and naltrexone on the serum concentration of IFN-γ (pg/ml) of the experimental animals (4 weeks after administration of alcohol, 3 weeks after administration of naltrexone), * $p< 0.001$; ** $p<0.0001$ vs control group, [a] $p<0.02$ vs group "alcohol".

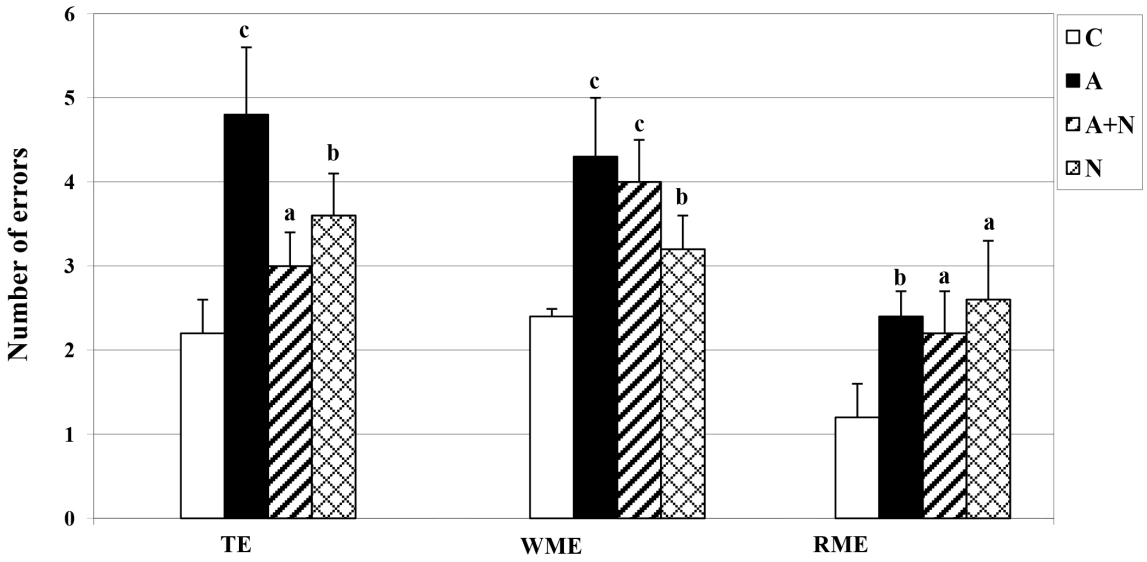

Figure 2. Effects of alcohol and naltrexone on the spatial memory in the 8-arm radial maze (2 weeks after alcohol, 1 week after naltrexone treatment). [a] $p< 0.01$; [b] $p<0.001$; [c] $p<0.0001$ vs control group.

significantly lower number of TE compared to the "alcohol" group ($p<0.0001$). In comparison with the control rats, the "naltrexone" experimental rats had a considerably higher number of TE ($p<0.001$), WME ($p<0.001$) and RME ($p<0.01$). The "naltrexone" group also demonstrated a decrease in the number of TE and WME errors in the 8-arm radial maze compared to the "alcohol" group ($p<0.0001$) (Figure 2).

Three weeks after treatment with naltrexone the TE number in "naltrexone" group was comparable to this of the control group (there was an insignificant difference). The "alcohol" group had significantly higher number of TE, WME and RME in comparison with the control group after 4 weeks of alcohol intake ($p<0.0001$). The group receiving alcohol for 4 weeks and treated with naltrexone for 3 weeks demonstrated no considerably different RME number, compared to the control one. In addition, these rats had significantly lower number of TE and RME compared to the "alcohol" group ($p<0.0001$). Group "naltrexone" had significantly decreased number of TE, WME and RME vs the "alcohol" group ($p<0.0001$) (Figure 3).

Discussion

Our results demonstrated that excessive alcohol intake decreased the production of IFN-γ in OVX rats. In addition, the administration of naltrexone for three weeks antagonized the effects of alcohol on IFN-γ production and increased its serum levels.

The present results are consistent with literature data for the role of estrogen deficiency on immune functions and IFN-γ levels in particular (Leiva-Revilla *et al.,* 2014;

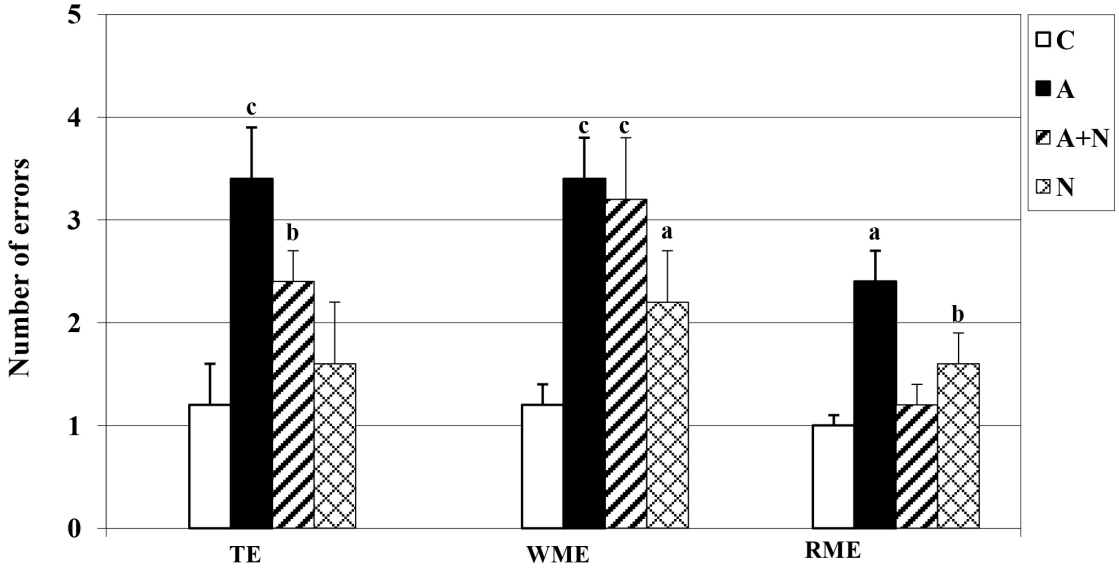

Figure 3. Effects of alcohol and naltrexone on the spatial memory in the 8-arm radial maze (4 weeks after alcohol, 3 weeks after naltrexone treatment).[a] $p < 0.01$; [b] $p<0.001$; [c] $p<0.0001$ vs control group

Xu *et al.,* 2015). OVX has been linked to increased susceptibility to infections, disrupted immune response and decreased serum IFN-γ (Rao *et al.,* 2015). Also, hormonal fluctuations are often associated with alcohol abuse (Epstein *et al.,* 2007) and chronic alcohol has been shown to substantially modulate immune functions (Franchi *et al.,* 2010).

Pharmacotherapeutic approaches are increasingly applied to enhance the efficacy of alcoholic remission. Naltrexone is an opioid antagonist that has been used for many years to treat alcohol dependence. Further, lots of recent reports demonstrate that naltrexone displays immunomodulatory effects (Nelson *et al.,* 2000; Ebrahimpour *et al.,* 2013) and it is a potential agent to attenuate immune deficiency in alcoholics (Jerrells *et al.,* 2007; Boyadjieva & Sarkar, 2010). Our current results support the previous findings and focus on the beneficial use of naltrexone in conditions with excessive alcohol intake and decreased estrogen levels.

Persuasive evidence has proved that estrogens promote neural cell proliferation, improve CNS function, and support neuroplasticity. OVX studies have demonstrated that the loss of estrogens is involved in morphological changes in cognitive centres (Su *et al.,* 2012) and learning and memory impairment (Talboom *et al.,* 2008; Minami *et al.,* 2013;). Chronic alcohol consumption is also associated with disrupted cognitive abilities (Vetreno *et al.,* 2011; Vedder *et al.,* 2015). Experimental data demonstrate that alcoholic rats show significant loss of performance in radial maze test (Gal & Bardos, 1994; Kuzina *et al.,* 1999).

In agreement with the abovementioned data, our current results showed that excessive alcohol decreased learning and memory functions of OVX rats. The present results also correlate with our previous report for the effect of OVX on cognitive functions and depressive-like behavior (Varadinova *et al.,* 2009). Furthermore, here we have demonstrated that chronic treatment with naltrexone improved spatial memory performance of OVX rats in the 8-arm radial maze. In the recent years substantial evidence has suggested the interrelation between the nervous and the immune systems. Campos et al, 2014 have proposed that IFN-γ is involved in CNS plasticity. Cytokines seem to contribute to the pathophysiology of neuropsychiatric disorders and cognitive functions (Lichtblau *et al.,* 2013). However, previous studies have gained inconsistent data about the role of IFN-γ in regulating emotion and intellectual-related functions (Maes, 2011; Kim *et al.,* 2012). Our data suggest an interrelation between the levels of IFN-γ and learning and memory abilities of OVX alcoholic rats.

It has been shown that naltrexone may improve cognitive functions but its cellular and molecular mechanisms remain unknown (Kibaly *et al.,* 2015). Our results propose that naltrexone enhanced IFN-γ levels and this might be related to the increased spatial memory capacity of the experimental rats.

The present data suggest that estrogen deficiency and excessive alcohol may have synergistic and adverse effects on immune factors and learning and memory abilities. Our findings address an intriguing interplay between OVX and excessive ethanol intake, which is associated with decreased levels of serum IFN-γ. In addition, we demonstrate that naltrexone reduces the harmful effects of our experimental models on immune and cognitive function. A better understanding of the underlying mechanisms may have a considerable role in preventing or treating their consequences. Furthermore, our results propose that naltrexone might have beneficial therapeutic effects on immunodeficiency and cognitive deficits in cases with estrogen insufficiency and alcohol abuse.

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