Research Article

RESVERATROL REVERSES THE RESTRAINT STRESS-INDUCED COGNITIVE DYSFUNCTION INVOLVING BRAIN ANTIOXIDANT SYSTEM IN RATS

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ABSTRACT

Objective:Restraint stress in rats is known to adversely affect the behavior especially memory dysfunctions in rats. Though there are many factors involved in this stress-induced memory impairment, damage to the antioxidant system in the brain is also known to be a causative factor. Resveratrol is known to exert its neuroprotective potentials by up regulating several antioxidant systems. Hence, the present study was undertaken to evaluate the neuroprotective effect of resveratrol against stress-induced cognitive impairment and involvement of brain antioxidant enzymes.

Methods: Male rats were subjected to restraint stress for 6 hours consecutively for 21 days. Another sets of rats received similar intensity and duration of stress along with either 10 or 20mg/kg dose of resveratrol for 28 days with its administration before a weak of stressing procedure. A control group of rats was also served in the experiment. The behavioural studies include active avoidance and open field tests. Thereafter the rats were sacrificed and the whole brain homogenate was subjected to lipid peroxidation and total antioxidants (TAO)estimation.

Results: Restraint stress has impaired motor activity, learning and memory activities and resveratrol treatment has reversed this cognitive dysfunction. Restraint stress caused an alteration in oxidative stress markers with a significant increase in lipid peroxidation and depletion of total antioxidant activities, which was reversed by resveratrol.

Conclusion: Results of the present study confirm that resveratrol can reverse the stress induced memory dysfunction by exerting its antioxidant potentials.

Keywords: Lipid peroxidation, Memory impairment, Restraint stress,Resveratrol, Total antioxidants.

INTRODUCTION

Stress induced neurotoxic effects are largely mediated by hypothalamic-pituitary-adrenocortical (HPA) axis with an increased brain corticosterone level[1] which intern affects expression of neurotransmitters, synaptic proteins and also affects the brain antioxidant defense system [2,3]. Stress induced neuronal damage associated with behavioral changes especially cognitive dysfunction is well reported [4,5].

The brain and nervous system are prone to oxidative stress, and are inadequately equipped with antioxidant defense systems to prevent 'ongoing' oxidative damage. Indeed, increased oxidative damage, mitochondrial dysfunction [6], accumulation of oxidized aggregated proteins is known to kill neurons. Restraint stress causes robust increase in the production of reactive oxygen species and consequent oxidative damage, with a concomitant decline in in vivo antioxidant defenses contributing to the pathology of stress-induced neurotoxic effects. Stress can alter cellular homeostasis by oxidative damage in brain which in turn involves behavioral, biochemical and morphological changes. Restraint stress in mice has been shown to cause neuronal death in the cerebral cortex which was prevented by antioxidant pretreatment with an associated decrease in reactive oxygen species population [7]. Antioxidants which effectively enter the nervous system, act as antioxidants themselves and are well absorbed from the GI tract would be ideal candidates for protection of the brain and nervous system from oxidative damage.Hence in the present study the antioxidant potential of resveratrol was evaluated.

Resveratrol is a phytoalexin produced by several plants that is sold as a nutritional supplement. It has also been produced by chemical synthesis [8]. A number of beneficial health effects, such as anti-cancer, antiviral, neuroprotective, anti-aging, anti-inflammatory and life-prolonging effects have been reported in non-human species (e.g. rats).Resveratrol was reported effective against neuronal cell dysfunction and cell death, and this theory could help against diseases such as Huntington's disease [9] and Alzheimer's disease [10]. In addition resveratrol decreased anxiety and increased cortex/hippocampus dependent memory of animals subjected to blunt head trauma [11]. Resveratrol is able to cross the blood brain barrier and exerts potent antioxidant features [12]. With many supportive results we tested the neuroprotective effect of resveratrol against stress induced memory dysfunction.

MATERIALS AND METHODS

Animals

Four months old male *Wistar* rats (weighing 220g±20) bred in-house was used in the present study. Animals were maintained under controlled conditions of light (10h- light: 14h- dark), temperature (22±3°C), and humidity (approximately 50±10%). All rats were maintained on the standard rat food and water ad libitum. For housing the rats' plastic cages with paddy husk as bedding material was used. The institutional animal ethical committee has approved this research protocol.

Stressing procedure

Rats were assigned to a daily restraint stress for 21 days in a wire mesh restrainer for 6 hours [13]. The wire mesh restrainer had a wooden base and stainless steel wire mesh restrainer hinged to the base. The restrainer with dimensions of 11cm (L) x 8cm (B) x 8cm (H) was used to stress. This type of restrainer will only restrict the movements of the animal without causing any pain, discomfort or suffocation.

Animal groups (n=6)

Group 1: Control and received sodium carboxymethylcellulose as vehicle

Group 2: Received 21 days restraint stress (6h daily)

Group 3: Received 21 days stress + resveratrol (10mg/kg body weight dose) for 28 days (Resveratrol was given a week prior to stress treatment)

Group 4: Received 21 days stress + resveratrol (20mg/kg body weight dose) for 28 days (Resveratrol was given a week prior to stress treatment)

Chemicals: Resveratrol (Cat. No. 70675) was obtained from Cayman Chemicals, USA. All other chemicals and reagents were HPLC or analytical grade (Sigma, St. Louis, Mo.)

Behavioral studies

1. Open field test

Open-field test is one of the most widely used methods to assess the motor and exploratory activities and emotional reactivity of rodents [14].

Apparatus: A rectangular box (100x100x40 cms) with the floor consisting 25 equal squares (5x5 cms) of fine unit wire mesh was used. Illumination is provided with 100 watts bulb fixed 60 cm above the centre of field.

Procedure: The rats were placed in one corner of the chamber. The number of peripheral and central crossings in a-ten-minute duration was recorded. Rearing (elevated hind limb & pelvis with elevation of fore limb) and grooming (use of head, tongue and fore limb for the process of cleaning various part of the body) activities were recorded. The open field exploration was assessed after completion of active avoidance test (thirteen days after last stress treatment).

2. Active avoidance(condition avoidance test /Shuttle box test)

The shuttle box consisted of a closed wooden box with shutters in front. The floor area was made up of grids, which were separated into two parts by a median grid. Each part was connected to separate electric circuits and a buzzer was fixed inside the box. Individual rats were allowed to explore the test box for 5 min. After 10 seconds, a discriminative stimulus was given through a buzzer. During that period, the rat could avoid the shock by crossing to the other compartment. If the rat failed to respond during the discriminative stimulus period, it received a shock of 2.5mA for a maximum period of 10 seconds, during which time it could escape by crossing to the other side. The contingency for avoiding the shock was a single crossing over the median grid from one side of the shuttle box to the other. The test consisted of 30 trials daily for 5 consecutive days. The number of shock avoidances increases from day 1 to 5 of the test under normal circumstances. The average shock avoidance numbers on all 5 days were termed as the mean score during 5 days of testing. Any decrease in this score is an indication of learning impairment. Each rat was retested one week after the last trial to assess retention of memory. A comparison of rat's performance with its previous performance gives the assessment of memory and was presented as the retention score (RTS).

$$RTS = \frac{Mean\ of\ retest\ score \times Mean\ scoring\ during\ 5\ days\ of\ testing}{Mean\ score\ during\ day\ 5\ of\ the\ testing}$$

Any decrease in the retest score and retention score is an indication of memory impairment. Each rat was trained for 4 days before starting the test [15].

Antioxidant studies

Measurement of Lipid Peroxidation

The level of lipid peroxides in brain supernatant was measured as thiobarbituricacid-reactive substances and determined using the method as described by Buege JA et al.,[16]. One milliliter of supernatant was precipitated with 2.5ml of ice cold tricholoroacetic acid (TCA). The sample was centrifuged at 3000g for 10min. To 2ml of this supernatant, 0.67% of thiobarbituric acid was added and kept in boiling water bath for 10min and cooled it. Malanodialdehyde (MDA) has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give pink chromogen, which was read immediately at 532 nm using a Systronic-117 UV-Visible spectrophotometer.Thiobarbituric acid-reactive substances (TBARS) concentration was calculated using molar extinction coefficient of chromophore $(1.56 \times 10^5 (mol/l) - 1cm\ -1)$ and the values were expressed in µmoles/gm protein.

Total protein concentration of tissues was measured by the method of Lowry et al.,[17].

Estimation of total antioxidants (TAO)

Total antioxidant activity in the brain was determined according to the method described by Koracevic et al.,[18]. A standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton - type reaction, leading to the formation of hydroxyl radicals. These reactive oxygen species degrade benzoate, resulting in the release of thiobarbituric acid-reactive substances (TBARS). The brain homogenate, capacity to suppress this TBARS formation was designated as the total antioxidant activity of the brain. The rate of inhibition of color development is proportional to the concentration of anti-oxidative activity. The fall in the absorbance was read spectrophotometrically at 535 nm. The total antioxidant activity was expressed as antioxidant activity in mmol/lit.

Statistical Analysis

The data were expressed as mean ± SD. The significance of differences among the groups were assessed using one way analysis of Variance (ANOVA) test followed by Bonferroni's multiple comparison test. Pvalues < 0.05 were considered as significant.

RESULTS

Behavioural studies

1. **Open field test**

Stressed rats made significantly (p<0.001) fewer central squares entries compared to control. Administration of resveratrol significantly (p<0.001) reversed (at both 10&20mg/kg dose) this effect by increased central square entries. Both grooming and rearing activities were also reduced significantly (p<0.001) in stressed rats compared to the control group. Resveratrol treatment at both doses in stressed rats has significantly (p<0.01) increased grooming activity but not rearing activity. The results of this study demonstrate that restraint stress adversely affects the motor activity and also emotional activity, but these toxic effects were reversed by resveratrol treatment (Fig.1).

2. **Active avoidance test**

The mean score during 5 days of testing, the retest score and retention score decreased significantly (p<0.001) in stressed rats compared to the control. Resveratrol at both the doses has increased these scores significantly (p<0.001) when compared with rats received only stress. The result of this study demonstrates that restraint stress has adversely affected the memory function but resveratrol treatment has reversed these neurotoxic effects (Fig.2).

Antioxidant studies

1. **Lipid peroxidation**

MDA level is a marker of lipid peroxidation. A highly significant (p<0.001) elevation in the expression of lipid peroxidation in the rat brain homogenate who received stress was noticed when compared to the control rat brain homogenate. Resveratrol at both doses has reduced significantly (p<0.001) the lipid peroxidation level when compared with stressed rat brain homogenate. Resveratrol at 10mg/kg dose had more profound effect compared to 20mg/kg dose (Fig.3).

2. **Total antioxidants**

A highly significant (p<0.001) reduction in TAO was observed in rat brain homogenate of the stressed rats when compared to the control rat brain homogenate. Resveratrol at both the doses has enhanced the TAO level, but it was highly significant at 10mg/kg dose (p<0.001) and moderately significant (p>0.05) at 20 mg/kg dose when compared to the stressed rats. There was also a significant difference between the two doses of resveratrol treatment (Fig.3).
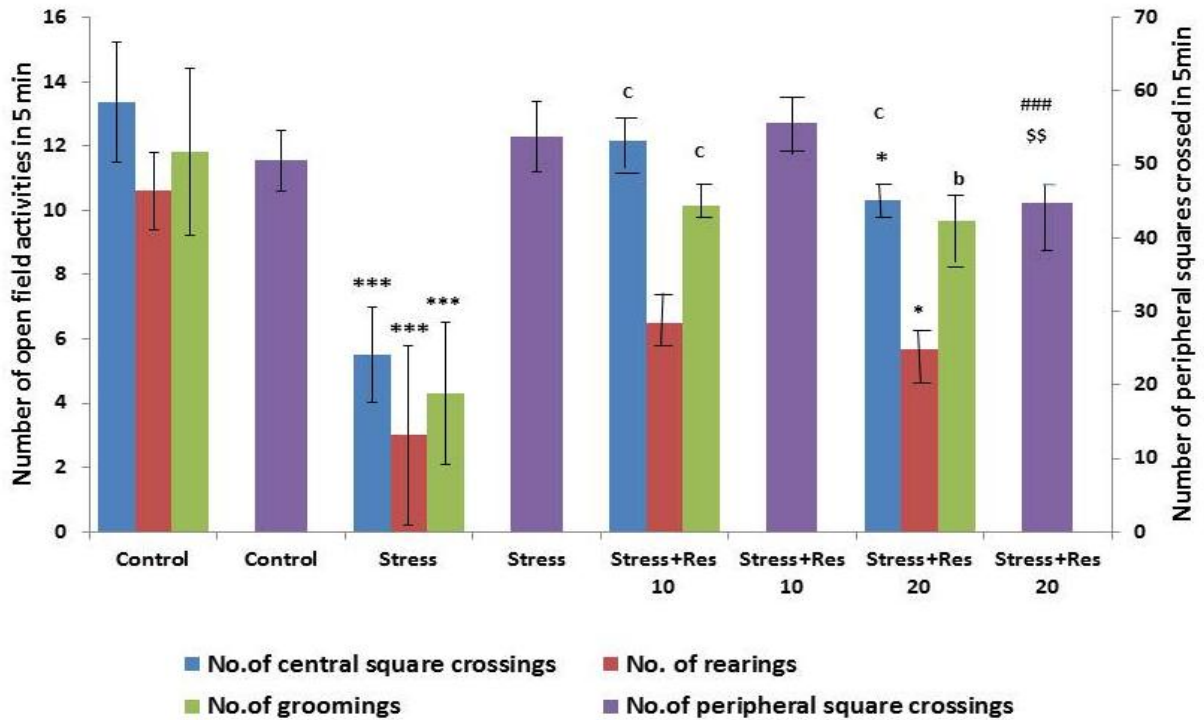
Fig. 1: Observation on open field activity by rats subjected to restraint stress and Resveratrol treatment.

Values are expressed as mean±SD (n=6). Comparision between Control Vs Stress for all parameters ***=$p<0.001$. Comparision between Stress VsStress+Res10 for all parameters, b=$p<0.01$, c=$p<0.001$. Comparison between Stress VsStress+ResV20 for all parameters, $$=$p<0.01$. Comparision between Stress+Res10 Vs Stress+Res20 for all parameters, ###=$p<0.001$. ANOVA significance-for central square crossed, F= 23.44, for peripheral square crossed, F=9.42, for groomings, F=14.52 and for rearings, F=8.38.
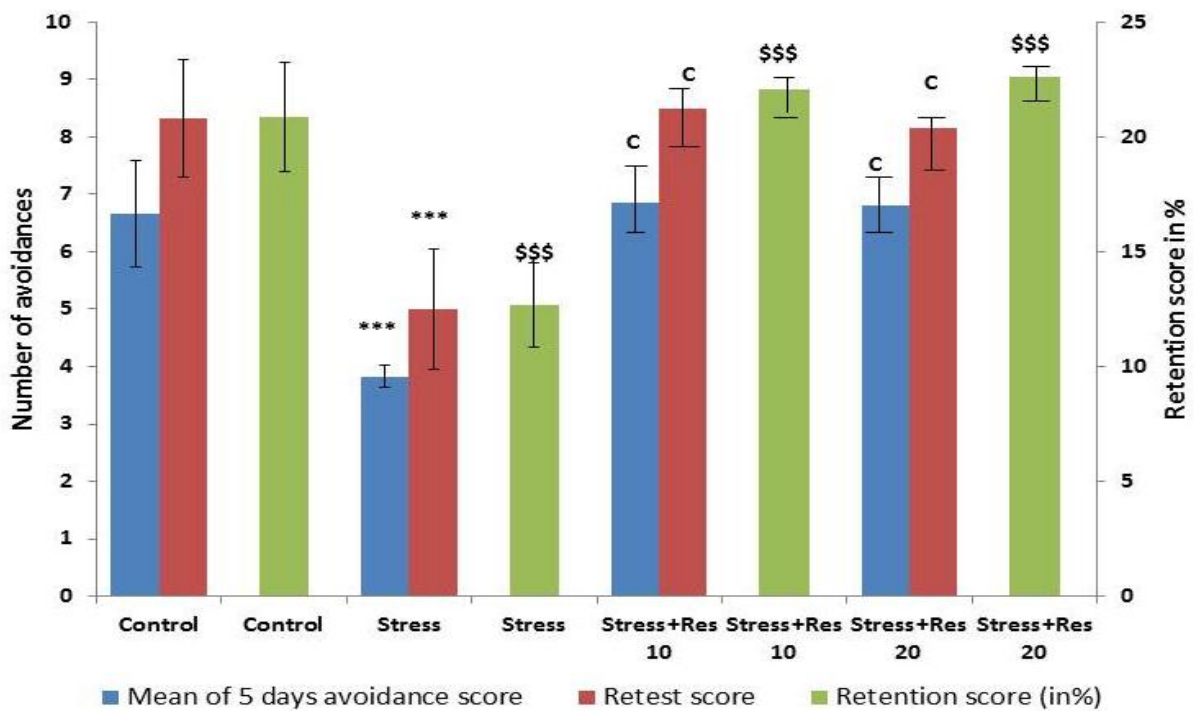

Fig. 2: Observation on condition avoidance by rats subjected to restraint stress and Resveratrol treatment.

Values are expressed as mean±SD (n=6). For mean of 5 days score & retest score, comparison between Control Vs Stress ***=$p<0.001$, Stress Vs Resveratrol treatment c=$p<0.001$. Comparison for retention score %, $$$=$p<0.001$. ANOVA significance-for mean of 5 days score, F=40.184, for retest score, F=18.98 and for retention score in %, F=17.27
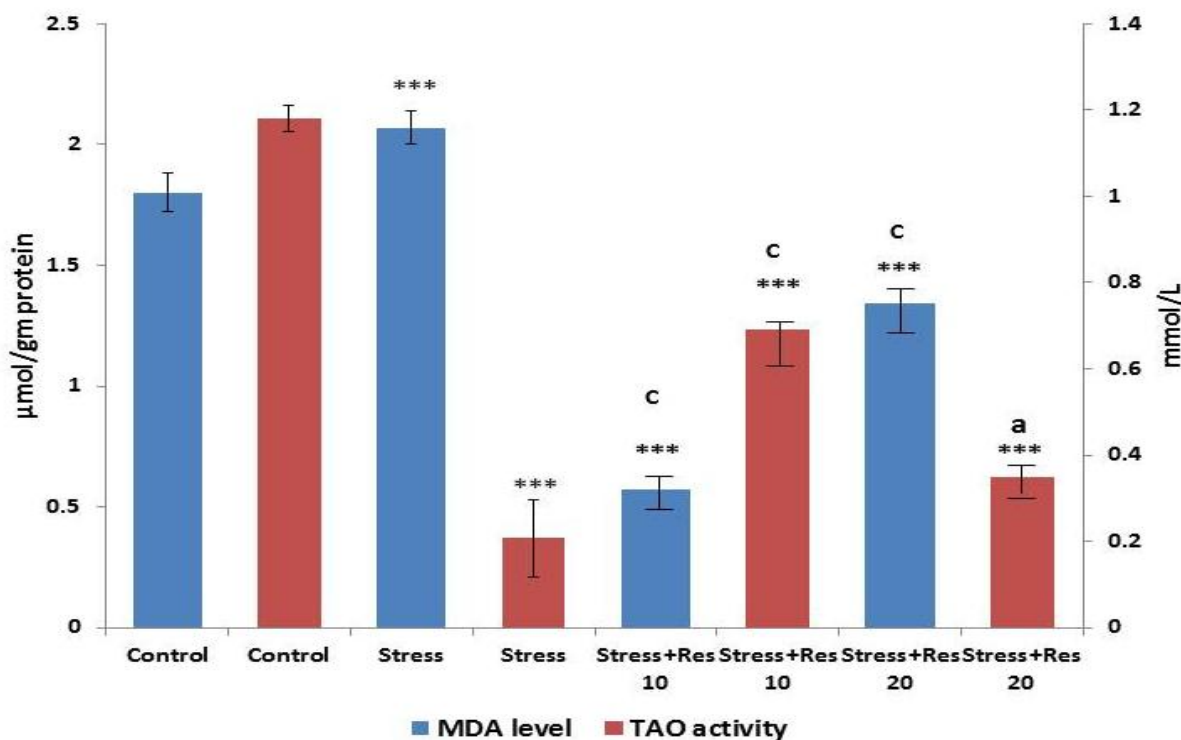
Fig. 3: Effect of restraint stress and Resveratrol treatment on antioxidant activities in rat brain homogenate.

Values are expressed as mean±SD (n=6). Comparison between Control Vs Stress, and Control with other resveratrol treatment groups, ***=p<0.001 for both MDA expression & TAO activity. Comparison between Stress Vs Resveratrol treatment groups, c=p<0.001, a=p<0.05 for both MDA expression & TAO activity. ANOVA significance for MDA level, F=425.78, for TAO activity, F=220.16.

DISCUSSION

The results of the present study clearly demonstrates that restraint stress in rats adversely affect the learning and memory abilities in rats. This result is consistent with number of reports published before [5]. Stress induced neurotoxic effects are largely mediated by hypothalamic-pituitary-adrenocortical (HPA) axis with an increased brain corticosteronelevel which intern affects expression of neurotransmitters, synaptic proteins and damage to the antioxidant defense system in the brain [19]. The present study was focused on stress induced memory impairment with respect to damage to the antioxidant system of the brain and whether resveratrol can reverse these effects. The results of the present study clearly demonstrates that restraint stresscauses oxidative damage to rat brain, as evidenced by significant rise in brain malondialdehyde (MDA – an endproduct of lipid peroxidation) levels, also significant reduction in total antioxidant activities.The consequence of oxidative stress to nervous tissue is many, as brain is particularly vulnerable to oxidativestress due to its high rate of oxygen consumption. Oxidativestress induces many damaging processes in stress disorderssuch as mitochondrial dysfunction, dysregulation of calcium homeostasis [20],disruption of energy pathway [21],damage to neuronal precursors, impairment of neurogenesis [22]. Restraint as a stress model combines both emotionaland physical components of stress. Exposure to chronicrestraint stress in rats, and psychological stress in humans, is implicated in the pathophysiology of mood and anxietydisorders.

Resveratrol was found to be a highly potent antioxidant that could inhibit free radical generation in the brain and the spinal cord [23,24]. It has been shown that it inhibits the lipid peroxidation [25] and prevents cell death induced by oxidative stress [26]. It has been postulated that resveratrol could suppress mitochondria-induced production of ROS in rat brain [27], protect DNA from oxidative damage in stroke-prone hypertensive rats [28]. NADPH oxidase-dependent production of superoxide radical has been identified as one of the major contributors to oxidative injury in the brain [29]. Resveratrol was shown to inhibit the activation of NADPH oxidase in different brain regions [30]. Lipid peroxidation in biological system has long been thought to be a toxicological phenomenon resulting in pathological consequences. MDA is very reactive and takes part in cross-linking with DNA and proteins, resulting in mitochondrial dysfunction and cell damage [31]. In the present study resveratrol treatment inhibited the MDA increase which was due to restraint stress. To support this protective effect, we further tested total antioxidant activity in brain homogenate.

CONCLUSION

Resveratrol treatment protected rat brain against stress induced oxidative damage. The results of this study will have impact in delivery ofbetter health care which is morefunctional and relevant in delivering the health care at the doorstep of thecommon man in the current scenario of stressful life style. Resveratrol could be a therapeutic strategy in stress-induced memory dysfunctions.

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