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Resveratrol improves postnatal hippocampal neurogenesis and brain derived neurotrophic factor in prenatally stressed rats

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ABSTRACT

Prenatal stress induced neuronal dysfunction is multifactorial, including suppressed neurogenesis in developing brain. Resveratrol is known to exert its neuroprotective potential by enhancing neurogenesis. But the efficacy of resveratrol against prenatal stress was not addressed in detail. Hence in the present study we evaluated the neuroprotective action of resveratrol on prenatal stress-induced impaired neurogenesis. Pregnant rats were subjected to restraint stress during early or late gestational period. Another sets of rats received resveratrol during entire gestational period along with early or late gestational stress. The study parameters included neuronal assay of doublecortin positive neurons (DCX +ve) and brain derived neurotrophic factor (BDNF) estimations in 40th postnatal day rat brain. Both early and late gestational stress resulted in significant decrease in generation of new born neurons and BDNF expression in hippocampus. The decrease in number of DCX +ve neurons and hippocampal BDNF expression was more profound in the offspring who received late gestational stress compared to early gestational stress. Resveratrol treatment has improved the expression of DCX +ve neurons and BDNF expression. These data suggest the neuroprotective efficacy of resveratrol against prenatal stress induced impaired neurogenesis.

1. Introduction

Life exists by maintaining a complex dynamic equilibrium that is constantly challenged by variety of intrinsic and extrinsic adverse stressors. It is well documented from human and animal studies that during the perinatal period, the development of an organism is subjected to complex environmental influences. Deleterious life events during pregnancy induce neurobiological and behavioral defects in offspring, some of them involving the hippocampal formation (Vallee et al., 1999; Lemaire et al., 2000; Reznikov et al., 2011). Prenatal stress indeed results in an enhanced production of stress hormones by the mother during critical periods of fetal brain development and provokes a definitively longer corticosterone response to stress in the offspring associated with a reduction in the number of hippocampal corticosteroid receptors (Vallee et al., 1999). Behaviorally, the progeny, from adulthood to senescence, exhibit memory deficits in a hippocampal-dependent task (Sahu et al., 2013; Vallee et al., 1999).

Recently, it has been hypothesized that hippocampal-mediated learning may be related to the generation of new neurons in the adult dentate gyrus (Elodie et al., 2005). These new neurons are produced from progenitor cells located in restricted brain regions, including the subgranular zone of the dentate gyrus of the hippocampal formation. Daughter cells, generated locally at the border between hilus and granule cell layer, migrate into the granule cell layer where they develop morphological and biochemical characteristics of mature neurons (Markakis and Gage, 1999). They receive synapses, extend axonal connections to CA_3 and become functionally integrated into existing neuronal circuitries (van Praag et al., 2002). It is well documented that prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus and this may be due to the altered hypothalamo-pituitary-adrenal (HPA) axis (Lemaire et al., 2000; Kawamura et al., 2006).

The exact role of new neurons in emotional regulation and mood state is not so clear. But several animal studies have indicated that adult offspring of prenatally stressed mothers show increases in affective-related behavior (Maccari and Morley-Fletcher, 2007; Weinstock, 2008) and decreased levels of hippocampal neurogenesis (Kawamura et al., 2006; Odagiri et al., 2008).

Network construction and reorganization, which are the main processes involved in neuroplasticity, are regulated by various factors. One of the factors associated with neuroplasticity is brain

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derived neurotrophic factor (BDNF). It plays an important role in the proliferation, differentiation, and survival of neuronal progenitor cells and even in hippocampal plasticity (Ramirez-Amaya et al., 2006). The BDNF gene is clearly regulated by stress and HPA axis activation (Yulug et al., 2009). Hence in our study we evaluated the effect of prenatal stress on BDNF expression in hippocampus. BDNF also supports neurogenesis and is required for the long-term survival of newborn hippocampal neurons.

Resveratrol (3, 4′, 5 trihydroxystilbene) is a naturally occurring phytoalexin present in high concentration in the skin and seeds of grapes (Soleas et al., 1997). Recently several animal studies have focused on the neuroprotective effects of resveratrol, showing, it slow down the neuropathology associated with Alzheimer's disease (Wang et al., 2006) and protect against damage to the brain due to trauma and cerebral ischemia (Della-Morte et al., 2009). Treatment with single dose of resveratrol after trauma significantly ameliorated the trauma induced hippocampal neuronal loss in rats. In addition resveratrol decreased anxiety and increased cortex/hippocampus dependent memory of animals subjected to blunt head trauma (Sonmez et al., 2007). Studies further extending to the level of neurotransmitters claim that resveratrol protects the dopaminergic neurons in the midbrain slice culture against multiple insults (Okawara et al., 2007). Monki et al. (2007) report that resveratrol is able to cross the blood brain barrier and exerts potent antioxidant features. Williams et al. (2009) reported that resveratrol is able to cross placental barrier and did not induce any adverse reproductive effects and mortality in an embryo-fetal toxicity study in rats.

Although mounting evidence convincingly demonstrates the neuroprotective and antioxidant activity of resveratrol in adult animals, the efficacy of resveratrol against prenatal stress was not studied with respect to neurogenesis and associated Brain derived neurotrophic factor (BDNF). This raised a critical question as to whether resveratrol can prevent prenatal stress-induced impaired neurogenesis.

To test this hypothesis, we first examined neuronal proliferation in progeny of stress-induced and resveratrol treated mothers with doublecortin (DCX), a microtubule-associated protein specifically expressed in migrating and differentiating neurons. This neuronal specific marker (DCX) was used to phenotype the newly born neurons. We also examined whether the resveratrol-induced changes in newborn neuronal cells in prenatally stressed was related to a change in BDNF level in the hippocampus. Several recent studies have examined functional dissociations between dorsal and ventral hippocampus. It has been reported that lesions of dorsal hippocampus, but not ventral hippocampus, caused spatial memory impairments in rats (Fanselow and Dong, 2010). Hence to correlate the prenatal stress-induced memory dysfunction with DCX & BDNF expression, we specifically selected the dorsal hippocampus.

2. Materials and methods

2.1. Animals and housing conditions

In-house bred male and female albino Wistar rats (3–4 months old) weighing 200–230 g were selected for the study. The rats were maintained in 12 h light and dark cycle in temperature and humidity controlled environment. The rats were fed with standard food pellet and water ad libitum. Polypropylene cage with paddy husk as bedding materials was used for housing the rats. Breeding and maintenance of the animals were done as per the guidelines of Government of India for use of Laboratory animals (Government of India notifies the rules for breeding and conducting animal experiments, proposed in the gazette of India Dec 15, 1998: which was reproduced in Ind. Journal of Pharmacol 31:92–95, 1999). Institutional Animal Ethics Committee (I.A.E.C) approval was obtained before the conduct of the study (IAEC/KMC/2010) and care was taken to handle the rats in humane manner.

2.2. Mating of rats and animal groups

Three female rats were allowed to mate with one fertile sexually active male rat for 4 h per day (separate male rats for each group). At the end of 4 h, female rats were separated and vaginal smears taken to detect the presence of sperm for the confirmation of pregnancy and the rats were designated as day 0 of pregnancy for further counting of the days. The pregnant rats were housed individually in separate cages with proper label indicating the day of conception and randomly allocated into six groups of six each. Three pregnant rats were used in each group. Two pups from each mother (one male and one female = total six pups) were considered for DCX study and again two pups from each mother (one male and one female = total six pups) were considered for BDNF study. The litter size was always more than 4 for each mother, to be included for both studies.

All the mothers delivered at term (22–24th day of gestation). The offspring were raised by their biological mothers until weaning (21 days after birth). The number of offspring considered for neonatal parameters is in accordance with Holson & Pearce (1992).

2.3. Stressing procedure

The pregnant rats were stressed (restraint stress) using a wire mesh restrainers (Madhyastha et al., 2008), thrice daily for 45 min (08:00 AM–11:00 AM, 12:00 AM–3:00 PM, and 4:00 PM–7:00 PM). The wire mesh restrainer has a wooden base with stainless steel wire mesh restrainer hinged to the base. A pad lock and latch is used to secure the rat in the restrainer. The restrainer with dimension 11 cm (L) × 6 cm (B) × 6 cm (H) was used for rats with gestation days 1–10. Restrainer of 11 cm (L) × 8 cm (B) × 8 cm (H) was used for rats with gestation day 11 to till delivery. This type of restrainer will restrict the animal movement without any pain, discomfort or suffocation.

2.4. Animal groups

Group 1. (Control) The pups belonging to the pregnant rats who received only 0.5% carboxy methyl cellulose in a dose of 10 ml/kg body weight (oral) throughout pregnancy.

Group 2. The pups belonging to the pregnant rats who received only resveratrol alone in a dose of 10 mg/kg body weight (oral) throughout pregnancy.

Group 3. The pups belonging to the pregnant rats who received restrain stress from gestation days 1–10.

Group 4. The pups belonging to the pregnant rats who received restrain stress from gestation day 11 to till delivery.

Group 5. The pups belonging to the pregnant rats who received restrain stress from gestation days 1–10 and resveratrol (10 mg/kg body weight, oral) throughout pregnancy.

Group 6. The pups belonging to the pregnant rats who received restrain stress from gestation day 11 to till delivery and resveratrol (10 mg/kg body weight, oral) throughout pregnancy.

Resveratrol (Cat. no. 70675, Cayman chemicals, USA) was obtained from Pro Lab marketing, New Delhi, India. The dose of resveratrol considered in the present study is according to the earlier study by Kumar et al. (2007).

2.5. ELISA analysis of brain derived neurotrophic factor (BDNF) level in hippocampus

The right or left hippocampus was carefully removed and rinsed with 1× phosphate buffered saline (PBS), homogenized in 1 ml of 1× PBS and stored overnight at −20 °C. After two freeze–thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 min at 5000 × *g*, 2–8 °C. The supernatants were used for ELISA analysis. BDNF levels were quantified using am commercially available ELISA kit (CUSABIO, catalog number. CSB-E04504r) according to the manufacturer's protocol. Briefly, 100 μl of samples and serially diluted BDNF standards (0–2000 pg/ml) were added to each well of ninety-six-well plates, coated with monoclonal BDNF antibody, incubated for 2 h at 37 °C. Liquid was removed from each well. 100 μl of biotin-antibody was added to each well and incubated for 1 h at 37 °C. Each well was aspirated and washed with wash buffer for 3 times using multichannel pipette auto washer. Then, 100 μl HRP-avidin was added to each well and incubated for 1 h at 37 °C. Each well was again was aspirated and washed as mentioned above. 90 μl of TMB substrate was added to each well and incubated for 15–30 min at 37 °C. 50 μl of stock solution was added to each well. Optical density of each well was determined within 5 min, using a microplate reader set to 450 nm. The level of BDNF protein in each sample was determined using the standard curve. The professional software "curve expert 1.3" was used to make the standard curve. Values were expressed as pg BDNF/100 mg tissue.

2.6. Immunohistochemical assay for doublecortin (DCX) in the hippocampus

On 40th postnatal day, female and male rats were used for immunohistochemical assay. Animals were sacrificed by cardiac perfusion with 4% paraformaldehyde under ether anesthesia. The brains were removed, postfixed in the same fixative for 48 h. Paraffin blocks were made in an embedding bath and blocked coronally and sectioned from the septal area where the two blades of the dentate are equal and formed a V-shape at post-Bregma. Sections of 7 μm were cut in the dorsal hippocampus using a rotary microtome (Jung Biocutt 2035, Leica, Germany) and sections were mounted on poly-L-lysine-coated glass slides. Immunohistochemical procedures for