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Protective effect of nebivolol on reserpine-induced neurobehavioral and biochemical alterations in rats

V.S. Nade *, N.V. Shendye, L.A. Kawale, N.R. Patil, M.L. Khatri

Department of Pharmacology, M.V.P.S. College of Pharmacy, Gangapur Road, Nashik, Maharashtra 422002, India

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ABSTRACT

Reserpine-induced orofacial dyskinesia is a model that shares some mechanists' aspects with tardive dyskinesia whose pathophysiology has been related to oxidative stress. The present study was aimed to explore neuroprotective effects of nebivolol, an antihypertensive agent, on reserpine-induced neurobehavioral and biochemical alterations in rats. Reserpine (1 mg/kg, s.c.) was used to induce neurotoxicity. Administration of reserpine for 3 days every other day significantly increased the vacuous chewing movements (VCMs), tongue protrusions (TPs) and reduced the locomotor activity in rats. Pre-treatment with nebivolol (5 and 10 mg/kg, p.o. for 5 days) showed dose dependant decrease in VCMs and TP induced by reserpine. Nebivolol also showed significant improvement in locomotor activity. Reserpine significantly increased lipid peroxidation and reduced the levels of defensive antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) in rat brain. Nebivolol reversed these effects of reserpine on oxidative stress indices; indicating amelioration of oxidative stress in rat brains. The results of the present study indicated that nebivolol has a protective role against reserpine-induced orofacial dyskinesia. Thus, the use of nebivolol as a therapeutic agent for the treatment of tardive dyskinesia may be considered.

1. Introduction

Treatment with neuroleptics in humans can produce a serious side effect, known as tardive dyskinesia (TD) (Busanello et al., 2012; Fachinetto et al., 2007). TD has promoted substantial research into the basic mechanisms of the efficacy and adverse effects of traditional neuroleptics (Casey, 2000). It involves a serious neurodegeneration which is associated with chronic exposure to neuroleptics in experimental animals and humans (Burger et al., 2003). Neisewander et al. have suggested that reserpine-induced oral dyskinesia may provide a new model for tardive dyskinesia (Neisewander et al., 1994). Though, the neuro-pathophysiology of tardive dyskinesia is not fully understood, it has been suggested that increase in striatal dopaminergic D_2 receptor expression is responsible for the onset of extrapyramidal side effects in humans and experimental rodent models of tardive dyskinesia. The dopaminergic hypersensitivity resulted as a consequence of proliferation of striatal dopamine D_2 like receptors leading to the long term dopaminergic blockade and has been suggested as a potential model for antipsychotic induced tardive dyskinesia (Vital et al., 1998).

Reserpine also causes depletion of brain catecholamines leading to an akinetic state in experimental animals. It has been observed that L-DOPA administration also alleviated the reserpine induced akinetic state, indicating that behavioral recovery is dopamine-dependant (Carlsson et al., 1957). The model of reserpine induced orofacial dyskinesia shows an important aspect of face validity with Parkinson's disease (PD) (Andreatini et al., 2002). It has been reported that repeated treatment with low doses of reserpine progressively induces alterations in motor function and an increase in striatal oxidative stress, indicating its application in the study of the neuroprogressive nature of the motor signs in PD (Silva et al., 2012). The decrease in glutamate uptake was observed in the subcortical parts of brain in animals treated with reserpine, indicating that early changes in glutamate transport may be related to the development of vacuous chewing movements in rats (Burger et al., 2005).

Burger et al. (2004) have reported that rats with vacuous chewing movements have significantly higher thiobarbituric acid reactive substances (TBARS) in striatum, indicating a rise in lipid peroxidation and free radical production associated with sharp reduction in activity of antioxidant enzymes like superoxide dismutase, catalase, glutathione reductase, etc. in such animals. Many researchers have tried to demonstrate that neurodegeneration involved in tardive dyskinesia is closely associated with generation of free radicals (Cadet and Lohr, 1989; Coyle and Puttfarcken,

* Corresponding author. Tel.: +91 253 2577250, mobile: +91 9922234934; fax: +91 253 2580250.
E-mail address: kawalevl@rediffmail.com (V.S. Nade).

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1993). The brain contains many antioxidant enzymes that prevent or suppress harmful free radical reactions (Dringen, 2005). It has been suggested that, the antioxidant treatment could be a key area in treating tardive dyskinesia in animal models (Abilio et al., 2002; Naidu et al., 2004). This particular model is advantageous because dyskinesia can be induced in short time span as compared to that induced by typical antipsychotics (Andreassen and Jorgensen, 2000).

Nebivolol is a third-generation β_1 adrenergic blocker with discrete pharmacological properties compared with other drugs exhibiting β blocking action (Kamp et al., 2010). One of the mechanisms of nebivolol's antioxidant activity is due to reduction of ROS produced by a NADPH oxidase system (Cominacini et al., 2003). Nebivolol has a direct scavenging activity on oxygen radicals with peculiar antioxidant properties, which may play a key role in various diseases (Mason et al., 2006). The objective of the present study was to investigate the neuroprotective effect of nebivolol against reserpine-induced orofacial dyskinesia in rats and to unravel its mechanisms action with respect to biochemical imbalances.

2. Materials and methods

2.1. Materials

Nebivolol hydrochloride (Hetero Labs, Hyderabad, India), vitamin E (Merck Ltd, Goa, India), reserpine (Research Lab, Mumbai, India), thiobarbituric acid (TBA) (Research-Lab Fine Chem Industries, Mumbai, India), nitrobluetetrazolium chloride (NBT) (Himedia Laboratories Pvt. Ltd. Mumbai, India), 5,5′- dithiobis (2-nitro benzoic acid) (DTNB) (Alfa Aesar, A Johnson Mathey Company). Bovine serum albumin (Spectrochem Pvt. Ltd., Mumbai, India). All the chemicals used were of analytical grade and purchased from standard manufacturers.

2.2. Animals

Male Wistar strain rats (150–200 g) were used for the study. Animals were housed in polypropylene cages and maintained under the standard laboratory environmental conditions; temperature 25 ± 2 °C, 12: 12 h L: D cycle and 50 ± 5% RH with free access to food and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. Each group consisted of five ($n = 5$) animals. All the experiments were carried out during the light period (08:00–16:00 h). The studies were carried out in accordance with the guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India). The Institution Animal Ethical Committee of M.V.P.S College of Pharmacy, Nashik approved the protocol of the study (IAEC/2013/01).

2.3. Experimental design

Animals were randomly assigned into 5 groups ($n = 5$ for each group). Group I – Vehicle (0.2% PEG (polyethylene glycol) in distilled water p.o + 0.1% acetic acid solution s.c, vehicle for reserpine), Group II – reserpine (1 mg/kg, s.c), Group III – vitamin E (10 mg/kg, p.o) + reserpine (1 mg/kg, s.c), Group IV – nebivolol (5 mg/kg, p.o) + reserpine (1 mg/kg, s.c), Group V – nebivolol (10 mg/kg, p.o) + reserpine (1 mg/kg, s.c), Group VI – nebivolol (10 mg/kg, p.o) alone to check its own effects on vacuous chewing movements and tongue protrusions.

2.4. Induction of orofacial dyskinesia

Vehicle treated group was administered with 0.2% PEG in distilled water orally for 5 days and with 0.1% acetic acid solution (vehicle for reserpine) subcutaneously for 3 days every other day. The first injection of acetic acid was given 24 h after the administration of PEG. Reserpine group received 1 mg/kg reserpine for 3 days every other day. Nebivolol plus reserpine groups were administered with 5 and 10 mg/kg nebivolol orally for 5 days and with 1 mg/kg reserpine s.c for 3 days every other day. The first dose of nebivolol or PEG was administered 24 h before reserpine. Nebivolol was administered 30 min before administration of reserpine. Similar procedure was carried out in vitamin E treated group of animals. Behavioral assessments were carried out on fifth day after 24 h of administration of the last dose of reserpine.

2.5. Behavioral testing

To quantify the occurrence of oral dyskinesia on the test day, rats were placed individually into a small Plexiglas observation cage (30 × 20 × 20 cm) to score vacuous chewing movements (VCMs) and tongue protrusion frequencies. Animals were allowed 10 min to acclimatize to the observation cage before behavioral assessments were performed. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. The VCMs and tongue protrusion were defined as single mouth openings in the vertical plane not directed towards physical material and visible extension of the tongue outside of the mouth respectively. If VCMs or tongue protrusion occurred during a period of grooming, they were not taken into account. The behavioral parameters of oral dyskinesia were measured continuously for a period of 15 min. In all the experiments, the observer was blind to the identity of the animals (Burger et al., 2003).

2.6. Assessment of total locomotor activity by actophotometer

Locomotor activity is an index of alertness of mental activity as most of the drugs acting on CNS influence locomotor activity. It is measured with the help of actophotometer which operates on photoelectric cells that are connected with circuit with counter. Interruption of light beams as a measure of movements of rats in a cage has been used by many authors. When a beam of light falling on photocell is cut-off by the animal, a count is recorded. Locomotion was measured up to 10 min for each rat (Vogel, 2002).

2.7. Biochemical estimation

2.7.1. Dissection and homogenization

On the 5th day immediately after behavioral assessments the animals were killed by decapitation. The brain was removed, rinsed with isotonic saline and weighted. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction for catalase assay was obtained by centrifugation (Remi – C - 30, Remi Industries Ltd. Mumbai, India) of the homogenate at 1000*g* for 20 min at 4 °C; for other enzyme assays, centrifugation was at 12,000*g* for 60 min at 4 °C. A Elico biospectrophotometer-BL200 was used for subsequent assays (Naidu et al., 2003).

2.7.2. Catalase activity (CAT)

Catalase activity was assessed by the method of Luck (1971), where the breakdown of H_2O_2 was measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H_2O_2 phosphate buffer (0.0125 M H_2O_2) and 0.05 ml of supernatant of brain homogenate and the change in the absorbance was measured at 240 nm. The enzyme activity was calculated using the millimolar extension coefficient of H_2O_2 (0.07). The results were expressed as micro moles of H_2O_2 decomposed per minute per milligram of protein.