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Original article

Attenuation of stress induced memory deficits by nonsteroidal anti-inflammatory drugs (NSAIDs) in rats: Role of antioxidant enzymes

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ABSTRACT

Background: Repeated stress paradigms have been shown to cause devastating alterations on memory functions. Stress is linked with inflammation. Psychological and certain physical stressors could lead to neuroinflammation. Inflammatory process may occur by release of mediators and stimulate the production of prostaglandins through cyclooxygenase (COX). Treatment with COX inhibitors, which restrain prostaglandin production, has enhanced memory in a number of neuroinflammatory states showing a potential function for raised prostaglandins in these memory shortfalls. In the present study, potential therapeutic effects of indomethacin and diclofenac sodium on memory in both unrestraint and restraint rats were observed.

Methods and results: Two components, long term memory and short term memory were examined by Morris water maze (MWM) and elevated plus maze (EPM) respectively. The present study also demonstrated the effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on lipid peroxidation (LPO) and activities of antioxidant enzymes along with the activity of acetylcholinesterase (AChE). Results of MWM and EPM showed significant effects of drugs in both unrestraint and restraint rats as escape latency and transfer latency, in respective behavioral models were decreased as compared to that of control. This study also showed NSAIDs administration decreased LPO and increased antioxidant enzymes activity and decreased AChE activity in rats exposed to repeated stress.

Conclusion: In conclusion this study suggests a therapeutic potential of indomethacin and diclofenac against repeated stress-induced memory deficits.

Introduction

Stress is not a pathological state by itself but the continuous exposure to stressful event has been related to the onset, progression or outcome of many disease conditions [1]. Under chronic stress, the hypothalamic-pituitary-adrenal axis and central monoaminergic systems is stimulated for extended periods of time [2]. The continuous release of stress hormones has deleterious effects in brain that are attributed to the release of secondary mediators [3]. These mediators include free radicals and inflammatory markers which are produced as a result of various inflammatory responses [4]. One of the inflammatory responses includes cyclooxygenase (COX) pathways. Prostaglandin E2 synthesized by COX-2 enzyme plays a key role in the generation of inflammatory response [5,6]. Inflammation is the primary defense mechanism to restore the normal cellular state from the disturbed state that may occur in response to any pathogen or stressful stimuli. The stress response in central nervous system showed cell destruction and apoptotic events in different regions of brain [7,8]. Continuous activation of inflammatory actions in brain may lead to neuronal damage due to the overproduction of free radicals [9]. These generated free radicals react with membrane phospholipids producing lipoperoxides, hydroperoxides, and malondialdehyde (MDA), which in turn may modify membrane permeability and integrity leading to neuronal loss [10]. Evidence recommends that neuroinflammation is directly involved in memory impairment [11–14]. Cholinergic system is mainly affected in dementia and related disorders. The cholinergic anti-inflammatory pathway suggests that the release of acetylcholine inhibits the production of pro-inflammatory cytokines [15]. Cholinergic system has been shown to selectively disrupt due to neuroinflammation, therefore, increasing levels of stress-induced neuroinflammation interrupt cholinergic pathway and decrease its inhibitory role on pro-inflammatory mediators which may further exacerbate the pathological condition. It is suggested that the inhibition of neuroinflammation may lead to

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improvement in cholinergic neurotransmission which may have a therapeutic effect on memory impairment [16].

It is suggested that the increased pro-inflammatory molecules can be inhibited pharmacologically to prevent the shortfalls in memory [17]. Presently, the role of prostaglandins as mediators of inflammation has increased immensely and nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to inhibit enzyme cyclooxygenases and other pro-inflammatory mediators. The pharmacological inhibition of COX-2 activity or deletion of its gene results in neuroprotection. Inhibition of COX-2 activity showed prevention against membrane lipid peroxidation (LPO) [18]. This suggests that COX-2 enzyme may act as a pharmacological target to prevent oxidative stress and thus inflammation-associated memory loss. It has been reported previously that NSAIDs play an important role in delaying the onset of certain neurodegenerative diseases including Alzheimer's disease [19,20], dementia and certain form of neural plasticity [21]. It is thought that NSAIDs possess free radicals and reactive oxygen scavenging activity and thus reduce oxidative stress [22]. Therefore in the light of above reported findings, the present study was designed to monitor the role of NSAIDs in stress-induced memory deficits caused by increased inflammation.

Materials and methods

Animals

Locally bred male Sprague Dawley rats weighing 120–150 g were used in the study. Animals were caged individually under 12:12 h light: dark cycle (light on at 6:00 h) and controlled room temperature (25 + 2 °C) with cubes of standard rodent diet and water. Prior to experiments, animals were subjected to one week of acclimation period and to various handling procedures in order to nullify the psychological affliction of environment for reducing the novelty and handling stress. All animal experiments were approved by the institutional ethics and animal care committee and performed in strict accordance with National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised1985). All treatment and behavioral monitoring were done in a balanced design to avoid order and time effect.

Drugs and chemicals

NSAIDs (indomethacin and diclofenac sodium), hydrogen peroxide (H_2O_2) stock (35%) solution, thiobarbituric acid, nitro blue tetrazolium (NBT), and dithiobisnitrobenzoic acid were purchased from British Drug House (BDH, Dorset, UK). Hydroxylamine hydrochloride, acetylthiocholine, ethylenediaminetetraacetic acid and all other analytical grade reagents were purchased from Sigma Chemical Co. (St. Louis, USA).

Experimental protocol

Thirty six animals (n = 36) were randomly divided into two groups as unrestraint and restraint. Each group was subdivided into three as control, indomethacin and diclofenac sodium groups. Controls were injected with saline (0.9%) whereas rats of indomethacin (7.5 mg/kg) [23] and diclofenac sodium (5.0 mg/kg) [24] groups were injected intraperitoneally with respective drug for five days. Animals of restraint group were given 2 h repeated stress after saline and drug administration. Restraint groups were subjected to 2 h of stress in ventilated, closed plastic tubes that allowed only limited lateral movement [25]. Unrestraint groups remained in their home cage throughout the duration of the experiment. After 24 h following the fifth stress long term memory and short term memory were assessed using Morris water maze (MWM) and elevated plus maze (EPM) respectively. After behavioral analysis rats were decapitated by using guillotine method and their brains were removed from the skull within 30 s after decapitation (Fig. 1). All samples were stored at −70 °C until analyzed for biochemical assays.

Apparatus

Elevated plus maze test (EPM)

The EPM was used to assess short term memory in rats. The apparatus constructed of Perspex plastic with 4 arms of 50 × 10 cm area. The two enclosed arms had side walls of 40 cm high. The open and closed arms were connected with a central square (10 × 10 cm) to give the apparatus a plus sign appearance. The whole maze was raised 60 cm above the floor. The maze was placed in the same position throughout the experiment in laboratory where extra maze cues were there to help learning. The procedure and technique were same as reported earlier by Batool et al. [26]. In the training session rats were individually placed at one end of the open arm, facing away from the central platform and the transfer latency (time taken in seconds for the rat to move into one end of the closed arms with all four paws) was recorded. The cut off time during the training session was 90 s for the rat to explore the maze. The test session to evaluate the retention of memory was performed after 1 h. During test session transfer latency was observed. Significantly decreased transfer latency was taken as an index of improved memory.

Morris water maze test (MWM)

The MWM is a known, conventional cognitive test that requires an animal to use spatial learning and memory to find a hidden platform just below the surface of a circular pool of water and to remember location of platform from the previous trial [27]. MWM used in this study was a circular pool of water with a diameter of 45 cm, height 37 cm and depth of water was 12 cm. The pool was a metal cylinder painted white on the inner surface and the escape platform was also made of metal cylinder with flat metallic top having a surface diameter of 8 cm and is 2 cm below the surface of water during water maze training. The pool was filled with water (23 + 2 °C) and made opaque with milk in order to obscure the platform and to allow proficient tracking of the swim paths of the rats. In this study long term memory in terms of latency to locate the hidden platform was assessed. On the 1 st day four training trials were performed during which escape latency was monitored. After placing in the tank each rat was given 120 s to find and mount on to the hidden platform, if the rat positioned the platform it was allowed to stay on it for 10 s [28]. After 24 h of training test was performed to assess long term memory.

Biochemical estimations

Estimation of LPO was performed as described by Chow and Tappel [29]. LPO was expressed as μmol of MDA/g of brain tissue. The superoxide dismutase (SOD) was estimated by the method [30], based on the reduction of NBT to water insoluble blue

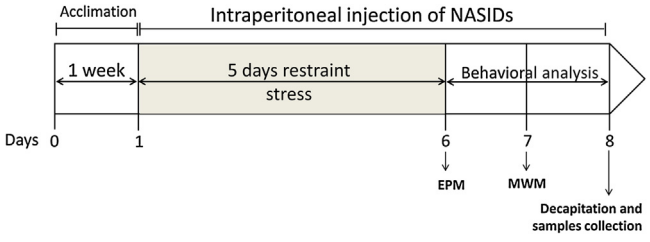

Fig. 1. Schematic representation of experimental protocol.