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Investigations in foot shock stress of variable intensity in mice: Adaptation and role of angiotensin II

Anjana Bali, Amteshwar Singh Jaggi*

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147002, India

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ABSTRACT

The present study investigated the stress adaptation and role of angiotensin in response to repeated exposures of electric foot shocks of varying intensity. Mice were subjected to moderate (0.5 mA) or severe (1.5 mA) electric foot shocks for 1 h for 5 days. Stress-induced behavioral changes were assessed by actophotometer, hole board, open field and social interaction tests. The serum corticosterone levels were measured as an index of HPA axis. Telmisartan (a selective AT_1 receptor blocker) was employed as a pharmacological tool. A single exposure of moderate and severe stress produced behavioral deficits and increased the corticosterone levels. The restoration of these alterations was observed in response to repeated exposures of moderate stress, while no adaptation was observed in severe foot shock stress. A single administration of telmisartan (5 mg/kg) exacerbated the moderate stress-induced decrease in behavioral activity and increase in corticosterone levels on the first day of stress exposure, suggesting the anti-stress role of angiotensin. In contrast, telmisartan normalized severe stress-induced behavioral and biochemical alterations suggesting the stress inducing actions of angiotensin. Furthermore, treatment with telmisartan abolished the stress adaptive response following repeated exposures of moderate stress suggesting that angiotensin has an adaptive role. It is concluded that there is a differential adaptive response in foot shock stress depending upon the severity of stress. Angiotensin II may act as an anti-stress agent and helps to promote the adaptation during medium stress, whereas it may promote stress response during severe stress.

1. Introduction

Stress is a state of threatened homeostasis during which a variety of adaptive processes are activated to produce a series of physiological and behavioral changes. Initial exposure to a stressor produces the behavioral alterations, including the reduced locomotor activity, decreased exploratory behavior, social withdrawal, etc. These behavioral changes have been attributed to the overactivation of hypothalamic–pituitary–adrenal (HPA) axis and sympathetic adrenomedullary system, which produce their effects by releasing glucocorticoids (corticosterone) and catecholamines (norepinephrine) into the blood, respectively (Bali and Jaggi, 2013). However, repeated exposure to a same stressor (homotypic) results in a diminished stress response in terms of the restoration of behavioral alterations and normalization of neuro-endocrinological changes, as compared to the initial stress response (Agrawal et al., 2011; Cohen et al., 1983; Kant et al., 1985). This blunted response to the stress stimulus during repeated exposure is referred as 'stress adaptation' and it has been suggested to be a key protective mechanism against repeated stress exposure (Rabasa et al., 2011; Stone and Platt, 1982; Stone et al., 1985).

Electric foot shock is a complex stressor, which includes both physical and emotional components. This stress paradigm comprises acute or chronic exposures of foot shocks of varying intensity and duration on the electrified grid floor in an electric foot shock apparatus (Armario et al., 1986, 1990; De Vry et al., 1993; Rabasa et al., 2011). Electric foot shock remains the most widely used stimulus for producing the measured amount of discomfort in animals due to its experimental advantage of control over the intensity and duration. Accordingly, scientists have developed foot shock-based disease models (Bali and Jaggi, 2015) including anxiety (Vogel et al., 1971), post-traumatic stress disorder (PTSD) (Louvart et al., 2005; Pawlyk et al., 2005) and depression (Seligman and Maier, 1967). However, unlike other stresses such as immobilization, restraint and cold immersion, the phenomenon of stress adaptation is not very well defined in response to electric foot shock (Rabasa et al., 2011; Stone and Platt, 1982; Stone et al., 1985). A number of studies have reported the development of adaptation in response to electric foot shock stress in terms of neural, endocrine, and behavioral responses in experimental animals (Ohi et al., 1988; Rabasa et al., 2011; Weiss

* Corresponding author. Mobile: +91 9501016036.
E-mail addresses: anjubali.123@gmail.com (A. Bali), amteshwarjaggi@yahoo.co.in (A.S. Jaggi).

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et al., 1975). However, studies also reveal the non-adaptive behavior in response to foot shock of variable intensity (Hajós-Korcsok et al., 2003; Van den Berg et al., 1998). The decline of the initial stress response after the repeated exposures to same stressor (homotypic) might result due to the release of endogenous neuropeptides. Indeed, the mechanisms responsible for the reduced unresponsiveness of stress system during repeated exposures of homotypic stress are known.

The renin–angiotensin system (RAS) is a complex, and is one of the best-studied enzyme–neuropeptide systems in the body. Apart from their well-documented presence in the periphery, the RAS components are also present in the CNS and play an important role in modulating the sensory, emotional and behavioral responses (Llorens-Cortes and Mendelsohn, 2002; Von Bohlen und Halbach and Albrecht, 2006). Research evidence suggests the critical role of central renin–angiotensin system in modulating the stress response as the different types of stressors (isolation, immobilization/restraint, cold restraint and immunological) influence the release of angiotensin II and expression of its receptors in the brain as well as in the peripheral tissues (Bregonzio et al., 2008; Yang et al., 1993). Preclinical and clinical studies have shown the effectiveness of the renin–angiotensin inhibitors in attenuating the stress-associated anxiety and improving the mood in depressed patients (Armando et al., 2001; Nasr et al., 2011). On the contrary, some studies have also addressed the anti-stress role of angiotensin neuropeptides (Hein et al., 1995; Ichiki et al., 1995). Furthermore, few studies have also described the role of angiotensin in cellular adaptive mechanisms in response to repeated neurogenic stress (McDougall et al., 2000). Therefore, the present study was designed to investigate the phenomenon of stress adaptation in electric foot shock stress and to identify the role of angiotensin neuropeptides in modulating stress adaptive behavior in response to repeated stress exposures of foot shock stress of varying intensity in mice.

2. Material and methods

2.1. Animals and drugs

Swiss albino mice weighing 25 ± 5 g were used in the present study and were procured from Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. Animals were fed on the standard laboratory feed and water. Animals were housed in the departmental animal house and were exposed to natural cycles of light and dark. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. no. CPCSEA/107/1999). Telmisartan was obtained as *ex-gratia* from Ranbaxy Pvt. Ltd., Gurgaon, India and suspended in 1% w/v of sodium carboxy methyl cellulose (CMC). BADGE (Bisphenol-A-diglycidyl ether) was purchased from Sigma-Aldrich, St. Louis, USA and suspended in 0.05% carboxymethyl cellulose (CMC). All the drug solutions were freshly prepared. The drugs were administered orally by intra-gastric gavage.

2.2. Acclimatization of animals

All mice were acclimatized for five minutes on the behavioral test apparatus for three days before initiating the actual experimental protocol. In behavioral studies, the acclimatization of animals to the test apparatus is necessary to avoid the potentially confounding effects due to the novelty of the test apparatus. Thus, acclimatization to test apparatus reduces the variation in the experimental data (Lapiz-Bluhm et al., 2008). The experimental procedures were done in between 8:30 a.m. and 12:00 p.m., when resting and stress levels of the HPA axis hormones are very stable (Rabasa et al., 2011). The blood sample was taken by a tail nick procedure under a resting condition to habituate the animal with the blood withdrawal procedure.

2.3. Induction of electric foot shock stress and stress adaptation

Mice were subjected to electric foot shock in a Plexiglas chamber ($26 \times 21 \times 26$ cm^3) with a grid floor made of stainless steel rods (0.3 cm diameter, spaced 1.0 cm apart) connected to a shock generator. After a habituation period of 2 min, a series of foot shocks of medium (0.5 mA) and high (1.5 mA) intensity of 1 s duration with foot shock interval of 4 min were delivered for 1 h to produce acute stress (Rabasa et al., 2011). Thereafter, mice were subjected to same electric foot shock stressor (homotypic stressor) of moderate and high intensity for five days to induce stress adaptation (Daniels et al., 2008; Van den Berg et al., 1998).

2.4. Assessment of stress and stress adaptation

2.4.1. Behavioral parameters

Ten minutes after the electric foot shock stress protocol, the battery of behavioral tests were performed in animals with the sequence of the actophotometer, hole board, open field, and social interaction tests with a time gap of 5 min between the successive behavioral tests. All behavioral test equipments were cleaned after each test with alcohol and water. The behaviors in the hole board, open field and social interaction were video-recorded and then analyzed.

2.4.1.1. Actophotometer test. The locomotors activity has been considered as an index of alertness and was assessed in the actophotometer. In an actophotometer, the movement of animals interrupts the beam of light falling on the photocell and a count is recorded digitally. Therefore, the number of counts is directly related to the movement of animals inside the actophotometer chamber. The animals were placed in the actophotometer for ten minutes and their activity was assessed in terms of count per 10 min (Agrawal et al., 2011; Kaur et al., 2010).

2.4.1.2. Open field test. The open field test has been employed to assess the stress-related behavior in rodents on the basis of changes in the exploration, general locomotor activity, and spontaneous activity (Prut and Belzung, 2003). Each mouse was exposed to an open field trial for 10 min in a dimly light room and each trial was recorded using a video camcorder positioned 2.1 m above the apparatus. Mice were placed in the center of the open field and the number of line crossings and rearings was noted (Agrawal et al., 2011).

2.4.1.3. Hole board test. The mouse was placed in the center of the hole-board and was allowed to explore the apparatus for 10 min. The animals were assessed for head dips and rearings (Agrawal et al., 2011; Kaur et al., 2010). The low number of head dipping reflects the high anxiety state level, and the number of rearing represents the exploration in the novel surrounding.

2.4.1.4. Social interaction test. The social interaction test has been widely used to assess the anxiety-related behavior. The social interaction test was carried out in the same box in which open field test was performed. During a 10 min test, each experimental mouse was allowed to interact with a partner mouse, which was socially housed and not subjected to any stressor. The behavior of a test mouse with test partner such as close proximity; facing and

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