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Protective effect of losartan and ramipril against stress induced insulin resistance and related complications: Anti-inflammatory mechanisms

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ABSTRACT

Chronic restraint stress (CRS) is known to cause various behavioural and biochemical alterations, leading to several negative health outcomes. The present study was designed to explore the impact of inhibiting Renin angiotensin aldosterone system (RAAS) and inflammatory pathways in stress pathophysiology. In the present study, male LACA mice were subjected to restraint stress daily for 30 days. Losartan, nimesulide, ramipril, minocycline and their combinations were administered 45 min prior to restraint stress daily and their effects were observed. Restraint stressed mice depicted depression like behavior along with increased oxidative stress markers in their brains. CRS induced insulin resistance depicted by hyperglycemia, hyperinsulinemia, hypercholesteremia, increased glycosylated hemoglobin and HOMA-IR. Besides, treatment with losartan, nimesulide, ramipril and minocycline significantly restored the behavioural and biochemical alterations and improved insulin sensitivity in stressed mice. Combination treatments synergistically reversed depression like behavior and decreased plasma glucose levels. Moreover they restored insulin levels, glycosylated hemoglobin levels and HOMA-IR values to the normal. This study signifies the synergistic effect of simultaneously blocking RAS and inflammatory pathways in stress pathophysiology.

1. Introduction

Stress, a state of threatened homeostasis is an integral part of today's human life and contributes to development of human psychopathologies like depression and metabolic abnormalities (Epel et al., 2004; Kessler, 1997). It leads to initiation of a series of adaptive changes in the body through activation of hypothalamic pituitary adrenal axis (HPA) and sympatho-adrenal system (SAS) (Axelrod and Reisine, 1984; Carrasco and Van de Kar, 2003). They in turn release adaptive hormones like catecholamines, corticosteroids and adrenocorticotropin triggering a multitude of autonomic, immune and behavioural responses collectively termed as stress syndrome (Nolan et al., 2015; Pardo et al., 2015). HPA axis is an important system that controls the stress response in integration with nervous system, secondary lymphoid organs of immune system and endocrine hormones (Hernandez et al., 2013) but sustained stress causes HPA axis fatigue and glucocorticoid resistance leading to heightened inflammation (Cohen et al., 2012; Miller et al., 2008). Studies report that depression is frequently comorbid with many inflammatory illnesses and increased inflammatory biomarkers (Almond, 2013). Moreover according to the cytokine hypothesis, depression is caused by a stress-induced increased production of proinflammatory cytokines which then induces oxidative stress led brain damage, impairing serotonin system and also contributing to the glucocorticoid resistance. The above hypothesis is supported by evidences in which excessive stress exposure induces damage to the brain, mediated through the neurotoxic effects of cortisol and neuroinflammation, which ultimately impairs neuronal plasticity (Stepanichev et al., 2014). Stress is also one of the emerging risk factor for metabolic disorders like insulin resistance and type 2 diabetes mellitus (T2DM) (Epel et al., 2004; Innes et al., 2007). Development of insulin resistance signifies the decreased ability of insulin to metabolise glucose and is characterized by hyperglycemia, a compensatory hyperinsulinemia, dyslipidemia, elevated blood pressure and abdominal obesity (Black, 2003). Increased catecholamines during stress increase renin release from kidneys by acting on β−1 receptors and is thus known to activate and potentiate renin angiotensin aldosterone system (RAAS). Circulating angiotensin II (Ang II) affects local brain RAAS and increase local Ang II concentration which then modulates T cell responses, stimulates the production of reactive oxygen species (ROS) by NADPH oxidase (de Cavanagh et al., 2007; Griendling et al., 1994). Ang II produced by activated RAAS also enhances the expression of chemokines, adhesion molecules and cytokines. Moreover reactive oxygen species is also involved in activating redox-sensitive inflammatory signaling pathways

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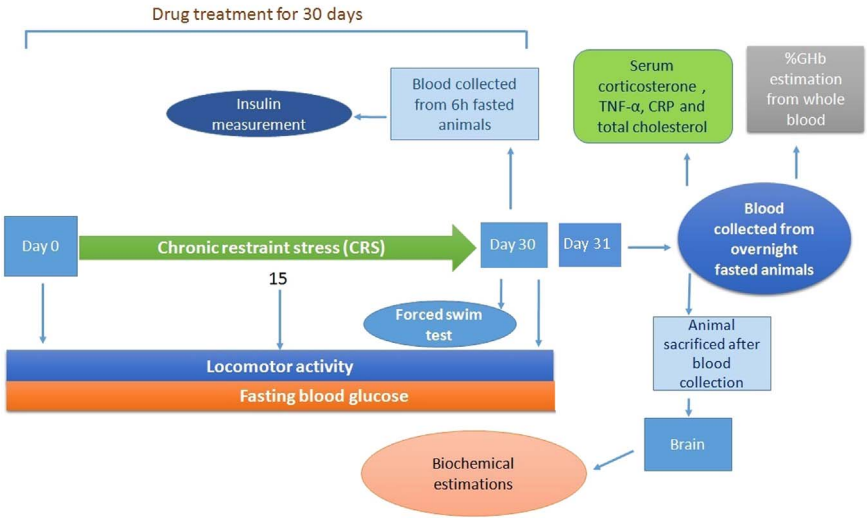

Fig. 1. Experimental protocol.

such as nuclear factor κB to increase production of proinflammatory cytokines, which induce phosphorylation of insulin receptor substrate-1 (IRS-1) at serine residues leading to negative insulin (Zhou et al., 2012). The RAAS is another neuroendocrine pathway which can be linked to the development of psychopathologies and insulin resistance via oxidative stress and activation of inflammation (Buren et al., 2003).

The present study was thus aimed to explore the influence of chronic stress on behavioural, biochemical and metabolic derangement in mice. Further the effect of drugs inhibiting RAAS pathway, inflammatory pathways and their combinations were studied for protective effect in above mentioned derangements.

2. Materials and methods

2.1. Animals

Male albino Laca mice (20–30 g, age 4–6 months) bred in the Central Animal House facility of Panjab University, Chandigarh, India were used for the study. The animals were housed in normal temperature (25 ± 1 °C) and humidity (45–55%) with alternate 12 h light and dark cycle. The animals had free access to water and standard rodent food pellets. They were acclimatized to the laboratory conditions before the experiment and all the experiments were conducted between 09.00 and 17.00. The experimental protocol was approved by the Institutional Animal Ethics Committee and performed according to the National Science Academy Guidelines for the use and care of animals (IAEC/415/434/17/09/13).

2.2. Drugs and treatment schedule

Losartan and ramipril dissolved in 0.9% saline solution were administered by intraperitoneal (*i.p.*) route and nimesulide and minocycline suspended in 0.25% w/v sodium carboxymethylcellulose (CMC) solution were administered by oral route (*p.o.*). Animals were randomly divided into following eight groups and received different drugs or vehicle daily for 30 days.

Group name	Treatment (mg/kg)
Naïve	Received vehicle
Control (RS)	Restraint stress (2 h daily) for 30 days
L (20)	Losartan (20 mg/kg)+Restraint stress (2 h daily) for 30 days
N (10)	Nimesulide (10 mg/kg)+Restraint stress (2 h daily) for 30 days
R (10)	Ramipril (10 mg/kg)+Restraint stress (2 h daily) for 30 days
M (100)	Minocycline (100 mg/kg)+Restraint stress (2 h daily) for 30 days
L (20)+N (10)	Losartan (20 mg/kg)+Nimesulide (10 mg/kg)+Restraint stress (2 h daily) for 30 days
R (10)+M (100)	Ramipril (20 mg/kg)+Minocycline (10 mg/kg)+Restraint stress (2 h daily) for 30 days

Drugs were administered 45 min prior to the animals being subjected to CRS. Doses were selected on the basis of previous reported studies (Danielyan et al., 2010; Kumar et al., 2010, 2015; Wiliński et al., 2007).

2.3. Chronic restraint stress

Animals were immobilized individually for 2 h at variable time period between 9:00 am to 5:00 pm daily for 30 days, in a modified restrainer comprising of properly ventilated, wooden box with dimensions of 7.5 cm length, 3 cm width, and 4 cm height with small opening at end through which tail of the mouse was kept fixed with zinc oxide hospital tape which further restricted the movement of animal. Finally mouse was released after moistening the tape with acetone in order to minimize pain or discomfort. The mice in naive group were kept in animal cage in the same experimental conditions. All the behavioural tests and biochemical estimations were done according to experimental protocol depicted in Fig. 1. Locomotor activity was performed one hour after CRS on day 0, 15 and 30 whereas FST was performed one hour after locomotor testing on day 30. The operators who performed the behavioural studies were kept blind to the treatments.

2.4. Behavioural tests

2.4.1. Locomotor activity

In order to assess the effect of CRS on locomotor activity, test for a period of 5 min was carried out using digital actophotometer [IMCORP, Ambala]. The actophotometer (30×30 cm) is equipped with infrared light sensitive photocells. Mice were individually allowed to