Food and Chemical Toxicology 47 (2009) 1239–1245

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Mechanism of action of *Rhodiola imbricata* Edgew during exposure to cold, hypoxia and restraint (C–H–R) stress induced hypothermia and post stress recovery in rats

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ARTICLE INFO

Article history:
Received 7 November 2008
Accepted 16 February 2009

Keywords:
Rhodiola
Cold
Hypoxia
Energy fuels
Glycolysis
Krebs's cycle

ABSTRACT

Mechanism of rhodiola root extract adaptogenic activity was studied in rats. The extract was orally administered in rats (100 mg/kg body weight), 30 min prior to cold (5 °C)-hypoxia (428 mmHg)-restraint (C-H-R) exposure up to fall of T_{rec}23 °C and recovery (T_{rec}37 °C) from hypothermia. In untreated control rats serum lactate and non-esterified fatty acids (NEFA) increased on attaining T_{rec}23 °C with decreased blood enzyme activities hexokinase (HK), phosphofructokinase (PFK), citrate synthase (CS) and glucose-6-phosphate dehydrogenase (G-6-PD), on attaining T_{rec}23 °C and T_{rec}37 °C. Decreases were also observed in liver and muscle tissues HK and G-6-PD enzyme activities and liver glycogen and CS on attaining T_{rec}23 °C and recovery; muscle PFK during recovery; muscle CS on attaining T_{rec}23 °C. Single and five doses of extract administration restricted increase in serum lactate values of rats on attaining T_{rec}23 °C and maintained blood NEFA in single dose extract treated animals, indicating improved utilization of NEFA as energy fuel. The single and five doses extract treatment decreased or better maintained tissue glycogen and enzyme activities, viz. HK, PFK, CS and G-6-PD, in blood, liver and muscle, on attaining T_{rec}23 °C and recovery. The results suggest that rhodiola extract treatment in rats shifted anaerobic metabolism to aerobic, during C-H-R exposure and post stress recovery.

1. Introduction

The living cells require adenosine tri-phosphate (ATP) to drive energy consuming biological processes. For meeting constant energy requirement of the cells ATP must be re-synthesized to sustain physical activity. In severe stressful situations like cold and hypoxia, organism requires higher rate of metabolism to cope with increase energy demand. Carbohydrates and fat are important oxidative energy fuels to meet the energy requirements of the organism. The maintenance of glycogen reserves and circulating glucose and free fatty acid levels are also required for meeting their enhanced utilization or energy production to sustain organisms in stressful situations. However, carbohydrate reserves of the organism are unable to provide energy for long duration due to their limited stores. Hence, body depends on other sources specially fatty acids for energy needs in situations such as fasting, stress and long duration exercises (Talwar et al., 1989). But at the same time the stress induced enhanced metabolism also results in increased generation of oxygen free radicals. Oxidative stress has been shown to reduce physical performance due to muscular damage (Hisao et al., 1993).

Stress responses are specific as well as non-specific. The stress producing factors are different and yet they all produce essentially the same biological stress (Selye, 1950). More energy is needed by the body during stress due to increased demand on body resources. To increase the energy demand and adapt during stress the hypothalamic–pituitary–adrenal (HPA) axis is activated. The HPA axis has been believed to play an important role in stress physiology and is one of the major hormonal systems mediating the stress response (Selye, 1950). The body responds to increased physical or psychological demands by releasing adrenocorticotropin (ACTH) from the anterior pituitary consequently glucocorticoids from the adrenal cortex, epinephrine from adrenal medulla and norepinephrine from sympathetic nerves. ACTH release is further regulated by

Abbreviations: ATP, adenosine tri-phosphate; cm, centimeter; C–H–R, cold Hypoxia restraint; EDTA, ethylene diamine tetra acetic acid; EMP, Embden–Meyerhof pathway; G, gram; HPLC, high performance liquid chromatography; Kg, kilogram; HK, hexokinase; HMP, hexose monophosphate; NADP, nicotinamide adenine dinucleotide phosphate; NEFA, non-esterified fatty acid; PFK, phosphofructokinase; CS, citrate synthase; G-6-PD, glucose-6-phosphate dehydrogenase; T_{rec}, rectal temperature.

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doi:10.1016/j.fct.2009.02.017

corticotrophin releasing factor (CRF) from the hypothalamus (Axelrod and Reisine, 1984). Hence, epinephrine, norepinephrine and cortisol a principle adrenal glucocorticoid in circulation in man and corticosterone in rats are important stress markers. These hormones in turn affect physiology and metabolism of the organism and during stress serve to adapt especially cardiovascular and energy producing systems. The natural products of herbal nature have been found to possess anti-stress activity. These substances act through HPA axis and anabolic processes to exert their anti-stress activity (Brekhman, 1980).

The management of unusual stress has acquired enormous significance in day-to-day life. It is possible to support the body's adaptation by using food supplements, dietary elements, herbs and minerals for increasing physical and mental performance. Such substances have been described as 'adaptogens' (Brekhman and Dardymov, 1969). Supplementation with various macro- and micronutrient and herbal preparations has been shown to possess adaptogenic activity during exposure to stressful environment (Kumar et al., 1996, 1999, 2000, 2002; Saggu, 2007). Rhodiola, a high altitude plant of family Crassulaceae, is widely distributed throughout Europe and Asia, with a reputation of stimulating the nervous system, decreasing depression, enhancing work performance, eliminating fatigue, and preventing high altitude sickness (Brown et al., 2002). Recently, roots of *Rhodiola imbricata* Edgew, growing on rocky slopes of western Himalaya (4000–5000 m), was found to possess radio-protective (Arora et al., 2005), cytoprotective and antioxidant (Kanupriya et al., 2005), wound healing (Gupta et al., 2007) and immunomodulatory (Mishra et al., 2006) activities. In a dose dependent study an aqueous extract of *R. imbricata* roots was found to possess potent adaptogenic activity in animals at a dose of 100 mg/kg body weight, during exposure to multiple stressful conditions of cold (5 °C)-hypoxia (428 mmHg)-restraint (C-H-R) and post stress recovery. The extract was also found to be safe in acute (LD_{50} > 10 g/kg) and sub-acute studies (Gupta et al., 2008). The studied aqueous extract of *R. imbricata* root was also reported to be free from heavy metal toxicity (Saggu et al., 2006).

The present study was carried out to examine the possible mechanism of adaptogenic activity of rhodiola dry root aqueous extract, administered orally in rats at a dose of 100 mg/kg body weight prior to C–H–R exposure (Ramachandran et al., 1990). The effect of rhodiola dry root vacuum dried extract's treatment was studied on circulating energy fuels viz. serum glucose, serum lactate and serum NEFA, stored glycogen reserves and status of some of the key metabolic regulatory enzymes hexokinase (HK), phosphofructokinase (PFK), citrate synthase (CS) and glucose-6-phosphate dehydrogenase (G-6-PD) in blood, liver and gastrocnemius muscle of rats, on attaining the T_{rec}23 °C during exposure to C-H-R and after recovery from C-H-R induced hypothermia (T_{rec}37 °C).

2. Materials and methods

2.1. Experimental animals

Male inbred Sprague-Dawley strain rats, 12–14 weeks old, weighing 150 ± 10 g were maintained under controlled environment in the institute's animal house at 25 ± 1 °C and 12 hour light-dark cycle. The animals were fed standard animal food pellet and water *ad libitum*. All the experiments were performed in accordance with the regulations specified by the Institutional Animal Ethical Committee (IAEC) and conformed to national guidelines on the care and use of laboratory animals, India.

2.2. Plant material

Rhodiola imbricata roots were collected in the month of September from Western Himalayas, India, where the plant grows widely under natural conditions at a height of over 4000 m. The Field Research Laboratory, Leh, where the voucher specimen of the plant material is preserved in the herbarium, carried out the ethanobotanical identification of the plant. The fresh roots were cleaned washed with water and re-washed with distilled water. Washed fresh roots were dried under shade in a clean, dust free environment and crushed using laboratory blender.

2.3. Extract preparation

Aqueous vacuum dried extract of rhodiola-dry root was prepared by cold percolation method as described earlier (Gupta et al., 2008). One gram of dried Rhodiola root produced 0.190 g of vacuum dried aqueous extract powder. The HPLC profile of the extract was studied and there was no batch-to-batch variation in the prepared extract (Gupta et al., 2008). The extract powder was suitably dissolved in distilled water to obtain the desired dose on body weight basis (mg/kg body weight) of the animals.

2.4. Experimental design

Overnight fasted 54 healthy male rats were used in the present study. The rats were divided into three groups with 18 rats in each group.

Group I. Untreated control;
Group II. Single oral extract dose (100 mg/kg) treated;
Group III. Five oral extract doses (100 mg/kg, single dose/day) treated.

The 18 rats included in each of the three above mentioned groups were further divided into three sub-groups with 6 rats in each sub-group.

(a) Rats not exposed to C-H-R.
(b) Rats exposed to C-H-R up to fall of T_{rec}23 °C.
(c) Rats exposed to C-H-R (T_{rec}23 °C) and recovered to T_{rec}37 °C.

The animals of untreated control group exposed to cold (5 °C)-hypoxia (428 mmHg)-restraint (C-H-R) were orally administered through gastric cannula 0.5 ml distilled water as vehicle, 30 min before exposure, while for unexposed rats 30 min before sacrifice. The second group of rats was orally administered a single oral dose of extract (100.0 mg/kg body weight) in 0.5 ml volume by gastric cannula, 30 min before C-H-R exposure. The rats of third group were administered by gastric cannula multiple oral doses (100.0 mg/kg body weight) of extract in 0.5 ml volume for five days (a single dose daily). Rats were exposed to C-H-R, 30 min after the fifth dose administration on day five. The unexposed animals, both single and five dose groups were administered 0.5 ml extract, 30 min prior to sacrifice.

2.5. C-H-R exposure and post stress recovery

The rats were exposed to C-H-R stress in a decompression chamber maintained at 5 °C and a low atmospheric pressure of 428 mmHg pressures equivalent to an altitude of 4572 m, as described earlier (Gupta et al., 2008). When the rats attained a rectal temperature (T_{rec}) of 23 °C, they were taken out of the chamber. The rats were allowed to recover to a normal T_{rec} of 37 °C at normal atmospheric pressure and ambient temperature 32 ± 1 °C. The time taken to attain T_{rec}23 °C and its recovery to T_{rec}37 °C were used as a measure of endurance (Ramachandran et al., 1990). The rats of the first sub-group of all the three groups of rats were not exposed to C-H-R, while the rats of second sub-group were exposed to C-H-R up to a fall of T_{rec}23 °C. The third sub-group of rats was exposed to C-H-R up to a fall of T_{rec}23 °C and recovered to attain T_{rec}37 °C.

2.6. Processing of tissues

After the experiments, animals of all the three groups i.e. control, single dose treated and five dose treated, were anesthetized by using anesthetic ether to stage of surgical anesthesia. Using capillary tubes blood samples were obtained from orbital sinus (Riley, 1960) in tubes with and without ethylene-diamine-tetra-acetic acid (EDTA), as per requirement. Animals were dissected and liver and gastrocnemius muscle tissues were quickly excised, washed in ice-cold saline and extraneous tissue material was removed. A 10% homogenate of the tissue was prepared using a Metrex tissue homogenizer (Metrex Scientific Instruments, Pvt. Ltd. Delhi, India) and sonicated (10 burst, for 5 s each interval) using a sonicator (IMECO Ultrasonics, Sonitron, Bombay, India). The assays of all the enzyme activities were performed at 25 ± 1 °C. The estimation of tissue glycogen and enzyme assays were carried out as described below.

2.7. Biochemical assays

2.7.1. Determination of circulating energy fuels

2.7.1.1. Estimation of serum non-esterified fatty acids (NEFA). Serum non-esterified fatty acid (NEFA) was determined by using Randox Kit, Cat. No. FA 115, 30t (Randox laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY). The purple color adduct formed by the peroxidase reaction was read at 550 nm in a Biorad spectrophotometer. Serum NEFA results have been expressed as mmol/l.