Screening Methods for Evaluation of Adaptogenic Agents: A Review

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ABSTRACT

Stress research in laboratory animals has assumed an important role in understanding the biological and behavioral consequences of external or internal stressors, which threaten to perturb homeostasis and may induce a number of clinical diseases when the body fails to counteract the stress situations. A variety of stress situations have been employed and the lack of consistency of the stress protocols is astounding2. The pharmacological assessment of adaptogens typically includes evaluation of their stimulating, tonic and stress protective effects in different screening models in which animals are challenged to acute and chronic stress conditions. Stress mediators and biochemical markers involved in mechanism of adaptogens may be evaluated using experimental procedures.

Key words: Adaptogen, stress, screening methods

INTRODUCTION

Stress disturbs the normal physiological condition and result in a state of threatened homeostasis. Stress has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases ranging from psychiatric disorders such as anxiety and depression, immunosuppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension and ulcerative colitis6. Stress research in laboratory animals has assumed an important role in understanding the biological and behavioral consequences of external or internal stressors, which threaten to perturb homeostasis and may induce a number of clinical diseases when the body fails to counteract the stress situations2. A variety of stressful situations have been employed and the lack of consistency of the stress protocols is astounding2. The pharmacological assessment of adaptogens typically includes evaluation of their stimulating, tonic and stress protective activities in different screening models in which animals are subjected to various stress conditions2. Despite considerable research effort, however, it still remains somewhat unclear which mediators of stress response are predominantly involved in the mechanism of action of adaptogens and which biochemical markers need to be assayed in the evaluation of drug efficacy.

SCREENING METHODS FOR ANTISTRESS (ADAPTOGENIC) AGENTS

Anoxia stress tolerance

Hypoxia is one of the most useful parameter for evaluation of Adaptogenic effect of a drug5. All the body functions, including cellular respiration depends on the oxygen supply. Any lack of vital element will play havoc on all body mechanisms and increase in adaptation during stress by any drug could be considered as its major antistress effect. During stress adaptogens are capable of increasing succinate dehydrogenase (SDH) in the brain. The enzyme (SDH) is responsible for utilization and conservation of energy in the cellular system of the organism; which helps adaptive processes during stress.

Groups of mice are treated for the period of 3 weeks. At the end of 1st, 2nd and 3rd week i.e. on 7th, 14th and 21st days 1 hr. after the treatment stress is induced to all the animals by keeping individually in hermetic vessel of 1 lit capacity. The time duration from the entry of the animal into the vessel and appearance of first convulsion is taken as time of anoxia tolerance6,7.

Swimming endurance test

Mice when forced to swim in a restricted space from which they cannot escape, become immobile after an initial period of vigorous activity. It has been suggested that the observed immobility signifies behavioral “despair” resembling a state of mental depression and has been used to screen anti-depressants. It is now recognised that this behavioral depression is fairly a common consequence of stress. It is also evident that the animal’s ability to cope with the stress largely influenced by the neurochemical consequence of stress. Thus exposure of animal to inescapable and severe stress leads to depletion of central nor adrenaline and serotonin, postulated to be the cause of endogenous depression8.

Animals under study are treated for 7 days. On 7th day 1 hr. after drug administration all the animals (mice) are subjected to swimming endurance test. End point of the test is death of the mouse due to drowning and swimming time of each mouse is noted. The mean surviving survival time for each group is calculated9.

Swimming and restraint stress induced gastric ulceration

Production of gastroduodenal ulcerations appear to be an inevitable consequence of stress, the intensity to be an inevitable consequence of stress, the intensity of the disease depending upon the duration of stress situation and appears to involve stress-induced auto-nomic and neuroendocrine system activation. As a consequence, both the offensive and defensive factors involved in the etiopathogenesis of peptic ulceration are affected10.

There are different methods for stress induced ulcers. In this test group of animals are treated for a period of 15 days. On 15th day 1 hr. after treatment all the animals are allowed to swim individually for 5 hrs. In restraint stress animals are exposed to immobilization at room temperature for 18 hrs. The effect of drugs on the severity and incidences of gastric lesions is observed11,12.

Cold-stress model

In the present study induction of stress is done using the cold stress. Animals are subjected to cold environment 4°C ± 1°C for 2 hrs daily for 10 days 1 hr after treatment. On 10th day serum glucose, cholesterol, triglycerides and BUN is estimated by sacrificing the animals. Weight of the organs such as liver, adrenal gland, spleen and testes are recorded after washing with alcohol with respect to their body weight i.e. 100 g

Gravitational stress

All animals (rats) are made to hang head down position from a horizontal bar daily 2 hrs. 1 hr. after treatment for a period of 8 days. Immediately after last exposure to stress, all the animals are sacrificed and the blood was collected for estimation of biochemical parameters like serum glucose, cholesterol, triglycerides and blood urea nitrogen (BUN). The weight of organs like liver, spleen and adrenal gland is recorded after washing with alcohol with respect to their body weight i.e. 100 g

Heat induced stress

All the animals (rats) are subjected to heat stress by exposing them to controlled temperature of 40 ± 2°C daily 1 hr. after the drug treatment for period of 8 days. Immediately after last exposure to stress, all the animals are sacrificed and the blood was collected for estimation of biochemical parameters like serum glucose, cholesterol, triglycerides and blood urea nitrogen (BUN). The weight of organs like liver, spleen and adrenal gland is recorded after washing with alcohol with respect to their body weight i.e. 100 g

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Noise stress
Noise as a stressful stimulus is a widely accepted fact. However an effective agent to counter the noise stress-induced biochemical alterations remains elusive.

Noise is produced by two loudspeakers (15 W), driven by white noise generator (0–26 kHz), and installed 30 cm above the cage. The noise level is set at 100 dB uniformly throughout the cage and monitored by sound level meter. Each treated animal is exposed for 4 hrs per day for 15 days and sacrificed on 16th day for study of effect of noise stress on central neurotransmitter levels i.e. norephernephrine, epinephrine, 5HT and dopamine.

Time taken to exhaustion (TTE) measurements
Animals are placed in a transparent bucket filled with water at ambient temperature and allowed to swim for 30 min. After this animal are dried and weight equivalent to 6 % body weight is attached to the tail and animals placed back in the water bucket. The time taken to exhaustion (TTE) was recorded as the time taken at which the animal stopped swimming.

C–H–R exposure
Exposure of organism to hypoxia and cold stress, a situation which exists at high altitude, produces neurohumoral and metabolic changes including oxidative damage24,25 which results in to mal-adaptation and decreased performance in such stressful situations.

The rats in overnight fasting state are restrained (creating emotional anxiety stress) in a decompression chamber maintained at 5°C. Rectal probes are inserted 2 cm past the rectum and retained there with the help of adhesive plaster. The rectal temperature (Trec) of the rats is monitored once per minute, by using a 16-channel Isothermex Temperature Recorder (Columbus Instrument, Columbus, OH). The rectal temperature of rats starts falling in the restrained state under cold and hypoxia. The time taken to attain a rectal temperature of 23°C is determined. The rat at this stage is taken out of cold-hypoxia chamber and is allowed to recover at normal atmospheric pressure and ambient temperature 32 ± 1°C in the restrained state. Again the time taken to recover the rectal temperature to 37°C is determined. The nature and shape of curve during fall of rectal temperature to 23°C and to recovery to 37°C along with the time is important indices, which is related to physical endurance and nature of the energy generation processes.

Biochemical analysis of blood like L lactate, glucose, free fatty acids, LDH, GSH, SOD etc are carried out26,27,28.

Anti-fatigue effect
Stress alters the normal functioning of the body29. In the special contrivance, when an animal forced to swim becomes immobile after an initial period of vigorous activity. This resembles a state of mental depression30,31 and causes severe fatigue.

Pre-trained mice which stayed on rotating rod (UGO Basile, Italy) at 20rpm, for more than 5 min. in three successive days, are used in this study. The grouped mice are given three successive trials to stay on rotating rod, on alternate days during the course of study. On day 15, on hr after treatment, animals of all the groups are exhausted by swimming continuously for two hrs. The animals are immediately taken out, dried with tissue paper and placed on the rotating rod to monitor anti-fatigue and motor coordination. The percent effect of each treatment is calculated on the basis of number of mice that stayed on the rotar rod for >180 seconds (by all or non method)32,33.

Stress induced analgesia
The endogenous pain inhibitory system can be activated by fear, anxiety, stress etc.34,35,36,37 It is well documented that stress activates different intrinsic pain inhibitory mechanisms, leading to analgesia in humans and antinociception in rodents, a phenomenon referred to as stress-induced analgesia/antinociception38.

The hot plate test used to measure changes in nociception response prior to and at different time points following a short (3 min) or long (15 min) swim exposure. Here the hot plate is used because it measures nociceptive responses at supra-spinal sites, where stress could cause the release opioi peptides39.

Carragaranean–induced edema in rats
Many exogenous chemicals and infectious organisms are known to induce trauma (stress) and this involves the leukocytosis, mediators of cell injury and blood vessels; as an result of which, normal physiological functioning and homeostasis of cell, organ and body as a whole is disrupted. Substance which reduce the state and severity of stress and counter the effects of stressors are adaptogens. The most prominent model used to assess such a type of antistress activity is carrageenan –induced truma in rats, which has been used extensively40.

Acute edema in one of the hind paw of rat is induced by the subplantar injection of freshly prepared 1% carrageenan suspension in normal saline. The edema is quanititated using plethysiographic recordings of paw volume before and after carrageenan injection41.

Chronic stress induced sexual behavior
Male rats are used for this paradigm. In rats of stress control group stress is induced by restraining the individual inside an acrylic hemi cylindrical plastic tube (4.5 cm diam. 12 cm long) for a period of 150 min once daily for 7 consecutive days. Male rat is placed in a cage for 10min with six oestronized sequentially treated with oestradiol valerate 5 mg/rat, followed 48 h later by hydroxyprogesterone 1.5 mg/rat, sc) female rats (120-150g), in a dimly light room. The parameters to be observed includes latency (in min) to lick female genitals, mounts and intromissions and the number of mounts and intromissions42,43.

Immune function
There has long been an interest in the role of stress in production of human diseased states, at least some of them being linked to suppression of the immune response. Stress is having adverse effect on normal immune surveillance44. The following methods are used

Cell mediated immune response
Rats are immunized with sheep RBC (SRBS) (1x106 cells sc) on the dorsum, on Day 1. Rats are challenged with 1x106 SRBC on Day 21, injected into left hind paw and saline is injected into the right hind paw. The differences in the footpad thickness (left-right) is determined 24hr later by mercury displacement technique45.

Humoral immune response
The peritoneal macrophage (PM) and PM phagocytosis assay technique is adapted for use in rats. PM is collected by washing the peritoneal cavity with 5 ml of Hank’s balanced salt solution (HBSS)+ bovine serum albumin (BSA) 10%. Fluid is recovered by gentle aspiration and aliquots (2x106 μl) are used for phagocytic assay. PM are incubated on glass plates (22x22 mm) at 37°C for 30 min in a humified chamber. The glass- adherent cells are incubated with live yeast cells of Candida albicans previously opsonized in autologous serum (5x106 in 600 μl of HBSS + autologous serum 50%) at 37°C for 30 min (digestion time), then washed with HBSS and again incubated for 30 min with autologous serum 50% (digestion time). The plates are with Wright and observed on a light microscope. A total of 300 cells are counted and the results expressed as phagocytosis percent, phagocytosis index and digestion index46.

Foot shock method
The method of Conti et al is adopted with some modifications required to add the element of unpredictability to the procedure. The rats are subjected to once daily 1-h footshock through a grid floor. The duration of each shock (2 mA) and the intervals between the shocks is randomly programmed between 3–5 and 10–110 s, respectively. Footshock stress is administered for 21 days47,48

Im mobilisation stress
Among the methods employed, immobilization has been extensively and accepted widely for studying the stress induced physical and psychological alterations and consequences of the stress. It is found to cause long-lasting centralization of hypothalamic-pituitary-adrenal axis (HPA) response which affects both peripheral and central components of HPA axis49. In this model rats are exposed to chronic stress daily for 2 hrs for a period of 10 days 1 hr after the drug treatment. Stress is induced by immobilising the rat with head down, supine position by fixing the animal to a board, inclined at an angle of 60°. The animals are sacrificed at the end of the last day immobilization. The adrenal gland, spleen and testes are also noted. Also blood levels of potential stress markers like NO, Cortisol, testosterone, prostaglandin and thromboxane etc are evaluated.

DIFFERENT STRESS MEDIATORS AND BIOCHEMICAL MARKERS
Cholesterol, BUN, triglycerides, glucose
The mechanism by which stress rises serum cholesterol is likely to be related to the enhanced activity of hypothalamo-hypophyseal axis (HPA) resulting in liberation of catecholamines and corticosteroids. This could lead to increase in blood cholesterol level19, since epinephrine is known to mobilise lipids from adipose tissues. The effect of stress on serum triglycerides19 has been shown to be variable. The increase in release of catecholamines40 leads to elevated levels of glucose49 and BUN40. In cold restraint and immobilization stress models, the test extract reduced the elevated levels of serum biochemical parameters in dose dependent manner.

Nitrone oxide
NO is short lived free radical that can be produced in mammalian cells by family of NO synthases (NOS), including neuronal (nNOS), endothelial (eNOS), and inducible (iNOD) enzymes, members of which have been shown to function as intracellular signaling regulators in a variety of cellular events50. Biological and/or physiological stress cause NO release in the body and modulate stress induced activation of the HPA axis and the sympathetic-adrenal medulatory system51. Endogenous NO can suppress JNK/SAPK through a thiol-redox mechanism52.

Cortisol
Cortisole hormone is known to be involved in the response to stress suppression in the immune system. Increased serum cortisol levels have been observed in connection with clinical depression and psychological stress involving stressors such as hypoglycemia, illness, fever, trauma, surgery, fear, pain, physical exertion or extremes of temperature. In normal release, Cortisol has widespread actions that help restore homeostasis after stress. It acts as a physiological antagonist to insulin by promoting gluconeogenesis, breakdown of lipids and proteins and mobilization of extra hepatic amino acids and ketone bodies. This leads to increased blood glucose concentration, resulting in increased glycogen formation in the liver53. In chronic stress, prolonged Cortisol secretion causes muscle wasting, hyperglycemia and suppresses immune/inflammatory responses.


Testosterone

Natural levels of testosterone decline gradually with age in men and also decline gradually with age in men and also decrease during stress. So adrenal gland weight increases. Cortisol increases mRNA levels in liver cells. This lead to increase in weight of liver. Spleen constricts to release more blood cells (RBC) during stress. So its weight decreases during stress.

THE IMPORTANCE OF STRESS DURATION

Acute or short- duration stress appears to have limited aversive effects on the individual since the body sets in motion an array of physiological, biochemical and endocrine responses to counter stress effects. However, chronicity and excessiveness of the stressor and the inability of the organism to cope with the stress appear to induce the syndromal state described by Selye in 1956. As such, a workable model of experimental stress has to incorporate the factors of chronicity, unpredictability and the inability to escape from the stressor.

CONCLUSION

Different stressors have different effects and there are differences in response to short- and long-term stress. Physiologically and chemically induced by stress the body. The main actors in this general adaptional response are believed to be the Hypothalamic Pituitary Axis (HPA) endocrine axis and the autonomic nervous system. Different stress mediators and biochemical markers are involved in the mechanism of adaptogens.

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