Volume 2, Issue 3, 2023, Page 262-271

International Journal of Pharma Research and Technology

Preclinical Models for Screening of Anti-Stress Agents: A Comprehensive Review

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Abstract

A stress response is activated when an uncomfortable stimulus is generated from an external environment or from within the organism, this activation leads to stimulation of the hypothalamic region of the brain which further stimulates and activates the pituitary gland, which leads to stimulation of the adrenal glands and release of stress hormones in the blood. This chain activation of the Hypothalamus, pituitary gland, and adrenal glands is the key step in the generation of the stress response. Various physical and psychological stress models are available for the evaluation of novel anti-stress agents. An ideal animal model should be capable of replicating the disease's natural development as well as each component of the stress response. The current review discusses various acute and chronic stress models, such as immersion in cold water, cold environment isolation, immobilization/restraint, forced swimming, food deprivation, neonatal isolation, predatory stress, day-night light change, noise, and so forth.

Keywords: Stress response, hypothalamus-pituitary-adrenal axis, physical stress, psychological stress, Immobilization stress, neonatal isolation, forced swimming.

Introduction

A stress response is a survival method that is a complex, dynamic process enhancing physical and mental conditions eventually affecting homeostasis.[1] It is the condition in which an individual undergoing stress reacts and responds physically, mentally, and emotionally to various stressful conditions.[2] Extreme stress can lead to severe forms of damage like degradation of dopamine neurons and failure to sustain homeostasis which may lead to fatal disorders including suppression of immunity, anxiety, anorexia, hypertension, heart failure, behaviour disorders like Parkinsonism, and hormonal imbalance leading to various endocrine disorders such as thyroid dysfunction.[3] Biochemical substances- 'neurotransmitters' are functionally entailed in the regulation of stress responses and are meant to contribute to resistance against stressful situations. This phenomenon is named adaptability. If the stress conditions are continued it impacts inefficient adaptation leading to decreased endurance or mood.[4]

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release of stress hormones in the blood. This chain activation of the Hypothalamus, pituitary gland, and adrenal glands (together called as HPA-axis) is the key step in the generation of the stress response.[5, 6] The external and internal stimuli also stimulate the sympathetic nervous system (SNS), all these activations are then seen to be compensated by physiological changes or acclimatization to the stress stimuli which will help in dealing with the danger portrayed by the stimuli. The activation of hypothalamic–adrenal axis and Sympathetic nervous system will cause the synthesis and release of glucocorticoid and catecholamines which in combination are known as ‘‘stress hormones’’.[7]

Stress has become such an inherent element of our everyday life that it is hard to imagine that the present-day usage of the term was introduced just a few more than 5 decades ago. Endocrinologist Hans Selye released a concept enunciating the term “stress” in the domain of health sciences in the 1930s.[8, 9] He observed that stress was associated with hypertrophy and atrophy of the adrenal gland and the thymus, lymph nodes, and spleen after the rats were administered with ovarian extracts. From his experiments on rats he showed that if the organisms exposed to non-specific nocuous agents such as acute exposure to cold, restraint, food deprivation, surgical injury, production of spinal shock, excessive muscular exercise such as in the case of forced swimming, or administering sub lethal doses of various drugs (epinephrine, morphine, formalin, etc.), a characteristic syndromes produced. Identical characteristic syndromes were also seen after administering the extracts from the placenta, pituitary, and spleen, as well as chemicals such as formalin. Hans Selye then coined the term “stress” and defined it as a “non-specific response of the body to any demand for change”.[10, 11]

The stress theory is composed of two major components: 1- Stressors: which can be of different types like physical (temperature, noise, light, restraint), biological (bacteria, virus, pollen-grains), chemical (formalin, adrenaline, cigarette smoke), and social/psychological (terror, human conflicts, unhealthy relationships) factors and 2- Stress responses: which includes secretion of the stress hormone, compensatory increased blood pressure, increased pulse rate, changes in blood glucose, etc. These stress responses are not certain of a specific type of stressor.[11]

Various pre-clinical animal models for the development of stress and evaluation of anti-stress novel drugs of natural, semi-synthetic, and synthetic origin have been designed and are employed often to assess the anti-stress activity of compounds from natural, semi-synthetic, and synthetic origin. A standard or an ideal pre-clinical animal model is expected to yield all the properties and conditions of stress in human beings and should reproduce the exact natural development of the disease in human beings.

Scientists and research workers from all over the globe have developed various preclinical animal models for acute and chronic stress which include forced swimming-induced stress, food, and water deprivation-induced stress, restraint-induced stress, immersion in cold water, cold environment isolation, day-night light change-induced stress, electric foot shock (EFS)-induced stress, neonatal isolation-induced stress, predatory stress, noise-induced stress, Cage tilt-induced stress, soiled cage-induced stress, etc.

Pre-Clinical Animal Models for Evaluation of Anti-Stress Agents

Animal models for evaluation of anti-stress agents can be broadly categorized into four types:

1. Physical stress models
2. Psychological stress models

Physical Stress Models

These models induce stress by subjecting the animals to physical distress and adversities. The physical stress model can be employed for acute and chronic studies based on the selection of aims and criteria by the researcher to assess the anti-stress activity of the novel compound. Most of the physical stress models are based on variations in body temperature. A switch in temperature causes stimulation of the thermoregulatory centre in the hypothalamus region and thereafter the Hypothalamic-Pituitary-Adrenal axis leading to stressful conditions which result in the secretion of adeno cortical hormones or stress hormones in the blood, which is believed to damage the temperature regulation capacity of the brain, consequently, animals are then unable to terminate the release of these stress hormones ultimately.[7]

Types of physical stress model:
a. Restraint stress
b. Immobilization stress
c. Temperature variation stress
d. Electric foot shock stress
e. Forced swimming induced stress

Restraint and Immobilization Stress

Immobilization or restraint as a stressor has been widely used for the evaluation of antistress agents by finally studying their effect on behavioural and biochemical responses in animals. Immobilization stress causes vascular oxidative stress by stimulating the angiotensin II/AT1 receptor signaling pathway, thereby triggering endothelial dysfunction which then results in the progression of atherosclerosis and hypertension.[12] Immobilization can be done in two ways:

a. Immobilization-induced stress with a restrainer: This stress model is rodents. Animals can be restrained in a semi-cylindrical acrylic tube (4.5 cm diameter and 12 cm long) with small air holes in it for them to breathe inside the restrainer.[13] Even though the animal's extent of movement is very restricted, the limbs are not restrained as such but the animal remains within a confined area. Because of this, the researchers have proclaimed that restraint stress is less profound than immobilization stress, and relative analysis of endocrine and neural factors has backed this proclamation.[7, 14, 15]

b. Immobilization-induced stress without restrainer: In this method, the animal's limbs are stretched on a board and immobilized with the help of adhesive tape. The head movements of the animals are restricted by keeping the head in a metal loop coiled around the neck portion of the animal. Immobilization of the rats once for 150 minutes is employed in the development of acute stress and immobilization for 7–10 days is used to produce chronic stress. Immobilization-induced stress can be induced by putting the animals under immobilization for different durations. This method subjects the animals to a complex stressor that comprises both physical as well as psychological dimensions in it.' Most frequently used model. The struggling, striving and muscular exertions of the limbs and neck that arise in the course of immobilization depict a powerful form of physical exercise. This method has the advantage that it develops an unavoidable physical as well as mental stress to which adaptation has been rarely shown.[16, 7] This model is one of the frequently used models for the induction of acute stress in rats.

Temperature Variation Stress

Alteration in the temperature causes stress in animals by stimulation of the thermo regulatory area of the hypothalamus and thereafter the pituitary and adrenal glands. This causes the adrenal glands to release stress hormones, leading to the acute stress response. A significant reduction in temperature by one of two - cold water or a freezer- can produce acute stress in laboratory animals.[17]

Mostly used methods of inducing acute stress by this model include the immersion of the laboratory animals in cold water (15-18.6 C for 15-30 min) or putting these animals in their cages and then putting the cages in a cold isolated environment (46 C for 15-30 min). These methods can be utilized for inducing both acute and chronic stress (7-14 days).[13, 18]

1. Immersion in Cold Water (ICW)

In the current approach, the rodents are set separately in a container filled with cold water (depth = 15.5 cm; temperature = 15-20°C) in which they might swim or just remain in an upstanding position with their head above the water's surface. This can be left immersed in this manner for 15 minutes unless the rats sink. In this case, rodents will be taken out of the container ahead of the cut-off time and those animals should not be incorporated into the study. Rats should be euthanized half an hour after exposure to stressors in the case of acute stress. In the case of chronic stress, rats can be exposed to stressors for 7-10 days. The acute stress model has the advantage that it can be accomplished in a short time when compared to the chronic stress model. Although, the chronic stress model has a significant disadvantage in that the animal adapts to the temperature changes due to long-term exposure to cold temperatures, and therefore the stress response is greatly reduced. The Immersion of animals in low-temperature water generates a marked increase in blood corticosterone levels, irrespective of the duration of exposure of animals to the stressors.[19, 20]

2. Cold environment isolation: In this model, rodents are kept at 4°C in a freezer for 15 minutes and 7-10 days for inducing acute and chronic stress respectively. This significant change or decrease in temperature causes a significant increase in the level of corticosteroids from the adrenal gland concluding in a stress response. Contrary to the cold-water immersion model, here rodents are not made to swim in the cold water and hence are saved from drowning and dying in cold water therefore it can be considered a comparatively safe model. Although this model has its limitation in that the animals get resistant when exposed to long periods of time.

There are many modifications to this method, one modification is that the rodents can be exposed to the stress by positioning them in their cages and then in a refrigerator at 8 degrees Celsius for 4 h. Rodents are exposed to this condition only once and their behavior is noted in the entire the stress experiment coupled with body temperature monitoring. However, this also has the same limitation of the development of resistance to chronic exposure.[13, 21, 22]

Electric Foot Shock-Induced Stress

Many researchers have used the mild intensity of Electric foot shock (EFS) as a stressor. Rodents are very sensitive to even mild electric shocks and display quick stress responses. Differing degrees of electric shock has been used by researchers to generate stressful conditions to evaluate the anti-stress activity of several compounds. Stress by this method is induced by positioning the rats separately in a compartment or chamber with an electrical grid floor supplied with power. Rodents are then exposed to unavoidable electrical foot shocks with a potency of 3 mA for a

period of 200 ms and a frequency of 1 per second over a 5-min duration. Rodents are exposed only once and euthanized after 15 minutes in the case of acute stress response. Whereas chronic stress is induced by repeating the procedure for 7-10 days after which rats are euthanized an hour after the last exposure.[13] A modified version of this method is also being used in which rodents are exposed to an unavoidable electric foot shock of 0.15 mA for 60 minutes (on different interval schedules with an average inter-shock interval of 60 seconds). The major advantage accompanying this model is that it efficiently induces elevated levels of stress in laboratory animals. The significant drawbacks of this model are the risk of mortality in animals due to the electric shock and the risk to the person performing the experiment, therefore this model should be performed by a skilled person taking extra caution.[13, 23, 24]

Forced Swimming-Induced Stress

The principle involved in forced swimming-induced stress is that animals have a tendency to avoid noxious conditions; if they are unable to avoid the stressful stimuli they feel vulnerable and thus exhibit a stress response. To induce stress with this method, rodents are allowed to swim in a cylindrical vessel (30 cm in diameter and filled to a height of 20 cm with 15 cm of space above the head of the rat) for acute stress a single session of forced swimming for 2 hours duration is used generally and for chronic stress one 2 hours session a day for 5successive days is used.[13, 25]

The forced swimming stress model is a considerably safe model, but adaptation due to long-term swimming in chronic swimming-induced stress has been stated and a difference in the behaviour of the animals in different strains has also been found.[26]

Psychological Stress Models

Fundamentally, this type of stress models produces stress by exposing the animals to psychological challenges. Animal models of stress that use psychological stress can be subdivided into:

- Neonatal isolation-induced stress
- Predatory stress
- Day-night light change-induced stress
- Noise-induced stress.

Neonatal Isolation-Induced Stress

Isolation of laboratory animals like rodents is done in special isolation cages, it is polypropylene cages covered with black paper from all four sides, but the roof is kept uncovered or exposed. The rodents are put individually in the cages for a particular duration of time as required for the experiment. In neonatal isolation stress models, maternal separation of the litter of the inbred strain is done. Typically, the neonate mice/rat is isolated from its mother on the second day after birth and kept in a different cage for a duration of 60 minutes away from the room where other animals are housed. Voices of other pups or other animals should be removed from the proximity of the isolated pups by playing a background white noise to have a masking effect. Isolation every day for 60 min and can be done on different days such as 4, 8, and 12 days.[27]

According to another source, the litter is taken out from the cage on the second day after the birth, they are weighed, and then placed separately in an opaque cage or any container (9 cm diameter and 8 cm deep) with no bedding for an hour (between 09:00 and 12:00) in a heated (30°C), a humidity-controlled chamber with white noise to obscure other pups' voice.[28-30] Initial life events profoundly affect the quality of succeeding years of life.[31] It has been demonstrated that the early life stress produced in neonatal isolation-induced stress in rats has instant and permanent neural and behavioural

effects.[31, 32] Effects like these may cause stress-induced structural changes in the hippocampus and other different regions of the brain.[33] Certainly, the hippocampus regulates the negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis.[34] and therefore neonatal isolation-induced stress model can depict the stress response that can result in neuro-degeneration at beginning of life. This stress procedure is efficient in determining the effect of stress on cognition and memory.[13]

These models aid in understanding the concepts of neural and behavioural effects generated due to stress and the consequences of these effects on quality of life at a later stage in life. This initial life isolation model is used in the study of alcohol or drug addiction.[27, 35]

Predatory Stress

If an animal is made to directly encounter its natural predator or their odours it will produce maximum stressful and antigenic states that the animal might develop 'flight or fight response.[36] During such conditions, there is a rapid activation of the sympathetic nervous system which will lead to a rise in the levels of adrenocortical hormones in the body further leading to the acute stress response.[37] A direct encounter with a predator has been efficiently used to evaluate the antis tress activity of various novel compounds by observing the biochemical and physiological changes produced. Predatory stress in mice is generally produced by a sequence of exposures to a natural predator like a cat or to any material possessing the odour of a cat like the faces of a cat.[38, 39] this model aids in studying post-traumatic stress-induced disorders (PTSD) in humans. Since there are various shortcomings while studying all the symptoms of PTSD during preclinical animal studies where invasive thoughts and nightmares cannot be tested. This model induces anxiety and hyper-arousal in the animal which is equivalent to PTSD in human subjects.[40, 41] There are shreds of evidence of chemical neurotransmitter changes in specific areas of the brain, for example- the amygdala, hippocampus, and prefrontal cortex which are associated with PTSD.[41]

In one of the methods suggested, mice are kept individually in separate cages for 20-min initially for environmental habituation. Each mouse is subjected to randomly offered 20-min predator confrontation sessions. Alterations inbehavioral patterns like locomotion activity, shrieking-like voices, and endocrinological changes after stress exposure can be observed.[42] Another free-exploration test can be done using a PVC box (30×20×20 cm) coated with Plexiglas and partitioned into 6 equal square units, which are interconnected by small entries.[43]

Parameters such as time spent in the novel compartment, total unit entries, and the total number of rearing are recorded. The result is expressed as the average percentage of time spent in the novel compartment, the average total number of novel unit changes, and the average total number of rearing. One of the major drawbacks of this model is the development of habituation to predator exposure therefore this model can only be used for inducing stress in only acute stress.[13]

Day-Night Light Change-Induced Stress

Circadian rhythm is regulated by the master clock present in the hypothalamic region of the brain. Alterations in the circadian rhythm can cause psychological and physiological imbalances.[44] In the current model, a reversal of circadian rhythm is created by artificial means by lighting the area of experimentation during night time. Rooms of animals are illuminated with lights during the night

from 7 pm to 7 am and darkness is maintained during the daytime. This reversal of the natural cycle will act as a stress stimulus but the procedure should not be repeated for long durations as there are chances of adaptation. This model is an excellent model for anxiety-related stress studies as the melatonin secretions from the pineal gland have an important role in the body in maintaining homeostasis.[44, 45] Melatonin is secreted by the pineal gland in response to dark or dim light whereas its functional antagonist serotonin is released in response to bright light. This serotonin-melatonin cycle regulates the sleep-wake cycle of the body.[46, 48] This method is mostly adequate for inducing short-term or acute stress responses. The induced stress can be evaluated by the measurement of biochemical parameters associated with the stress response. One of the major limitations of this model is that it can be only used to generate short-term stress responses, on repeated exposure to this type of stress stimulus adaptation to the new or changed day-night light cycle can occur. This disadvantage can be overcome by using this model as a part of chronic unpredictable stress protocol (CUS).

Noise-Induced Stress

Noise is a kind of stress stimulus and people living in urban cities are continuously exposed to motor- vehicles which emit noise at greater decibels than normal healthy levels. Likewise, occupational exposure with the people working in such industries is also common and noise is a hazardous psychological stimulus of stress. Various studies have shown ultra-structural alterations of mitochondria in rat cardiomyocytes due to noise stress. These intracellular modifications are linked to an imbalance in calcium homeostasis, which is believed to be sustained by enhanced catecholamine innervations in the noise-induced model.[49] A depletion effect on free radical scavenging due to noise has also been reported which causes a state of moderate to severe oxidative stress which can further lead to hearing loss.[50, 51]

Commonly, loudspeakers are used together with white noise. Usually, these speakers are placed about 30-40cm distance from the laboratory animal's cages usually near the roofing of the cages with a noise limit of 100dB to higher levels. These animals can be exposed for short durations (acute stress) or repeated exposures (chronic stress) from 3 to 4 hrs period every day for a duration of 5, 10, 15, or 30 days based on the study plan[51-54]

Conclusion

Stress is a complex survival response affecting physical and mental well-being. Animal models play a crucial role in stress research and assessing anti-stress agents. Physical stress models involve subjecting animals to distressing conditions like restraint, immobilization, temperature variations, electric shocks, and forced swimming. Psychological stress models expose animals to challenges, such as neonatal isolation, predatory encounters, day-night light changes, and noise. These models help understand the physiological and behavioural effects of stress. Researchers must choose appropriate models based on study objectives. Understanding stress responses and developing effective anti-stress agents is essential in addressing the growing impact of stress on human health and well-being. In conclusion, the study of stress and the evaluation of potential anti-stress agents using preclinical animal models contribute significantly to our understanding of stress-related disorders and pave the way for the development of novel therapeutic interventions. Continued research in this field is crucial for improving human health and

enhancing our ability to cope with the challenges of modern life.

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