CHAPTER II
LITERRATURE REVIEWS

2.1 Stress and Stress Responses

All living organisms strive towards a dynamic equilibrium, which is called homeostasis. In the classical stress concept, stress is defined as a state of disharmony, or threatened homeostasis. This equilibrium is threatened by the physical and psychological events or intrinsic or extrinsic disturbing forces known as stressors, which can be either acute or chronic (Chrousos and Gold, 1992). When homeostasis is disturbed, a new homeostasis is re-established by various physiological and behavioral adaptive responses (Chrousos, 2009). The adaptive responses can be specific to the stressor or can be generalized and nonspecific (Table 2-1) chronic (Chrousos and Gold, 1992). Stress consists of three components including input of stimuli, evaluation of information and response output.

Table 2-1 Behavioral and physical adaptation during acute stress

Behavioral Adaptation	Physical Adaptation
Adaptive Redirection of Behavior	**Adaptive Redirection of Energy**
Increased arousal and alertness	Oxygen and nutrients directed to the CNS and stressed body site(s)
Increased cognition, vigilance, and focused attention	Altered cardiovascular tone, increased blood pressure and heart rate
Euphoria or dysphoria	Increased respiratory rate
Suppression of appetite and feeding behavior	Increased gluconeogenesis and lipolysis
Suppression of reproductive behavior	Detoxification from endogenous or exogenous toxic products
Containment of the stress response	Inhibition of growth and reproductive systems
	Inhibition of digestion-stimulation of colonic motility
	Containment of the inflammatory/immune response
	Containment of the stress response

(Adapted from Chrousos and Gold, 1992)

Stress response consists of the complex patterns of physiological, behavioral, cognitive, and/or emotional components (Steckler, 2005) which occur mediated by both central and peripheral systems. It has been reported that the stress related mediators in the central nervous system (CNS) are hypothalamic hormones, such as corticotrophin releasing hormone (CRH), arginine vasopressin (AVP), pro-opiomelanocortin-derived peptides and norepinephrine while the mediators in the peripheral system are included glucocorticoids, norepinephrine and epinephrine. The stress related systems receive and integrate a great diversity of neurosensory (higher cortical, limbic, visual, auditory, olfactory, gustatory, somatosensory, nociceptive, visceral) and blood-borne signals (blood composition signals, hormones, cytokines, other mediators) that arrive through distinct pathways (Chrousos, 1998) and exert their influences to regulate the brain's cognitive, reward and fear systems and wake-sleep centers as well as the growth, reproductive and thyroid hormone functions. Moreover, they also exert their influences on the functions of gastrointestinal, cardiorespiratory, metabolic, and immune systems. Therefore, the impairment of stress response can disturb growth, development, behavior and metabolism (Chrousos, 2009). The interaction between homeostasis disturbing stressors and stressor activated adaptive responses of the organism produces three outcomes including eustasis or a condition which an organism can return to its basal condition, cacostasis or the condition which adaptive response to stress is inappropriate either excessive or inadequate and the situation which the organism gains experience and a new, improved homeostatic capacity is attained (Figure 2-1) (Chrousos, 2009).

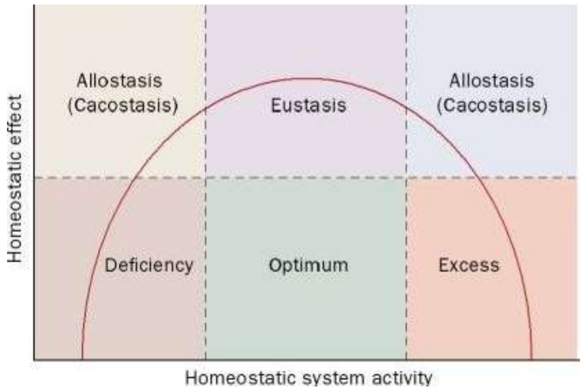

Figure 2-1 Homeostatic systems exert their effects in an inverted U-shaped dose-response curve (Chrousos, 2009)

Accumulating evidence also points out that stress responses primarily involve the activation of two main pathways two main pathways: the hypothalamic–pituitary–adrenocortical (HPA) axis and the sympathetico–adrenomedullary system (SAM). It has been reported that the first phase of stress response is attributed by the function of sympathetic nervous system via catecholamine whereas the second phase of this response is associated with glucocorticoids. It has been reported that psychological stress is commencing with the impulses arising from high cortical centers of the brain and relayed through the limbic system, results in the release of chemical mediators such as norepinephrine (NE), serotonin, and acetylcholine which in turn activate paraventricular nucleus (PVN) of the hypothalamus leading to the release of corticotropin releasing factor (CRF). CRF can stimulate the secretion of norepinephrine (NE) from central nucleus of amygdala and locus coeruleus. It has been reported that locus coeruleus is a dense collection of noradrenergic nerve fiber in brainstem (Reul *et al.*, 1998).. Therefore, the activation of this area plays a crucial role on the integration of autonomic function, somatosensory/pain processing, and mediating arousal. In addition, the stimulation of the sympathetic fiber also elicit the response of chromaffin cells, equivalent postganglionic neuron in adrenal medulla, giving rise to the release of epinephrine (E) (Black, 2002).

In addition to CRF, PVN also synthesizes arginine suppressing (AVP), the hormone playing the important role on the stress response amplification amplification (Whitnall, 1993). Both CRF and AVP are released into the hypophyseal portal system and stimulate the synthesis and release of pro-opiomelanocortin products (POMC) including both adrenocorticoteophic hormone (ACTH) and β-endorphin. To date, it has been reported that ACTH is the key regulator of glucocorticoids secretion from adrenaline cortex. The main glucocorticoid in rodent appears to be corticosterone whereas that in human is cortisol. Glucocorticoid, a final effectors of the hypothalamic–pituitary–adrenocortical (HPA) axis, play the crucial role on stress response (Habib *et al.*, 2001) (Figure 2-2)

The effects of glucocorticoids occur via two types of receptor including the mineralocorticoid receptor which has a higher affinity for corticosterone and the glucocorticoid receptor which possesses a lower affinity for corticosterone (Meijer and De Kloet, 1998; Meijer *et al.*, 1998). After binding to the receptor, glucocorticoids play a principal role on energy metabolism, growth processes, immune function and brain function, including learning and memory processes underlying behavioral adaptation (Chrousos, 1998; Gesing *et al.*, 2001; Stratakis and Chrousos, 1995; Stratakis *et al.*, 1995). Besides the aforementioned, glucocorticoids can also exert the influences on neuronal excitability (Reynolds and Hastings, 1995), neuronal plasticity, dendritic remodeling and neurogenesis (Sandi, 2007). Moreover, the activation of mineralocorticoid receptors in the hippocampus can also inhibit the activity of HPA-axis (Gesing *et al.*, 2001) in pyramidal neurons of Cornu ammonis (CA) and in granular neurons of dentate gyrus of the hippocampus (Gerlach and McEwen, 1972; Herman and Cullinan, 1997). Hippocampal neurons also send the projection to activate the inhibitory GABA-ergic neurons located in the ventrolateral septal region and the bed nucleus of the stria terminalis, which in turn project to CRF neurons in the hypothalamic paraventricular nucleus (Herman *et al.*, 1989). It has been shown that glucocorticoids play the important role not only on the function of nervous system but also on the regulation of HPA-axis by exerting the negative feedback at the levels of hypothalamus and pituitary gland (De Kloet *et al.*, 1986) and in some supra-hypothalamic structures (De Kloet *et al.*, 1986; Gesing *et al.*, 2001; Meaney *et al.*, 1996; Meijer and De Kloet, 1998; Reul *et al.*, 1998).

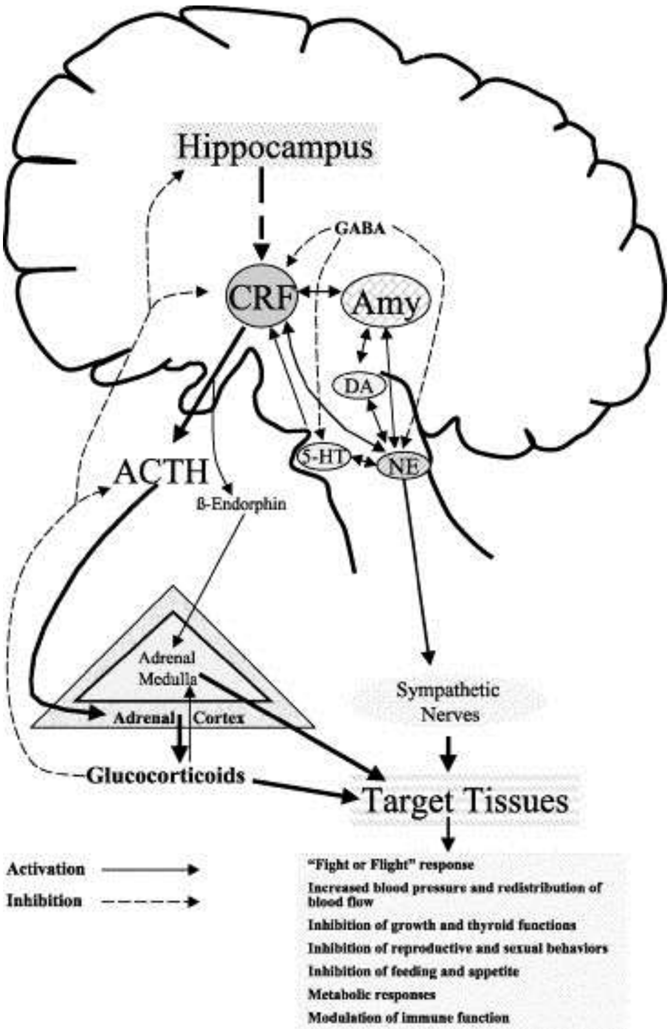

Figure 2-2 Brain circuits playing the role on the regulation of the neuroendocrine stress response. CRF = corticotrophin-releasing factor in the hypothalamic paraventricular nucleus; 5-HT = serotonin in the dorsal raphe nucleus; NE = norepinephrine in the locus coeruleus; DA= dopamine in the mesolimbic system; Amy = amygdala; GABA= gamma-amino-butyric acid (Carrasco and Van de Kar, 2003).

2.2 Memory

Memory is an organism's ability to store, retain, and recall information and experiences. Memory process comprises of 3 main processes including encoding, storage, and retrieval.

Encoding is the first step in creating a memory. It is the process by which information is transformed into a memory representation. This process is influenced by a number of factors, including attention and elaboration (Johnson and Hasher, 1987; Wickelgren, 1981). Recent imaging study shows that the encoding process of different types of memory involves different brain networks. It has been reported that the encoding of semantic memory which reflects general knowledge about the world involves left prefrontal cortex whereas the encoding process of episodic memory involves the function of hippocampus especially CA2, CA3 and dentate gyrus (Zeineh *et al.*, 2003). In addition, both mid and anterior regions of inferior prefrontal cortex also involved this process. Therefore, prefrontal cortex involves both semantic and episodic encoding processes (Prince *et al.*, 2007). The neurotransmitters involve the encoding are cholinergic, gamma amino butyric acid (GABA) (Hasselmo *et al.*, 1996) and glutamate (Winters and Bussey, 2005).

After the information is encoded, the memory trace is stabilized or consolidated (Dudai, 2004). Consolidation process can be classified into 2 specific processes consisting of synaptic and system consolidation. It has been reported that synaptic consolidation occurs within the first few hours after learning and involves long term potentiation (LTP). Several lines of evidence point out that LTP in hippocampus contributes an important role on the consolidation which change short term to long term memory of declarative memory, LTP in amygdala contributes an important role on the consolidation of emotional learning and memory (Maren, 1999). LTP in hippocampus appears to involve with the function of 3 pathways including 1) the perforant pathway, an excitatory connection between subiculum and granule cells of the dentate gyrus, 2) the mossy fiber pathway or the connecting fiber between granule cells of the dentate gyrus and pyramidal cells in area CA3 of the hippocampus 3) Schaffer collateral pathway connects the pyramidal cells of the CA3 region with the pyramidal cells in the CA1 region of the hippocampus (Kandel, 2001).

Glutamate (Glu) and NMDA receptor are crucial for LTP (Tsien, 2000). After being released, Glu will activate both AMPA and NMDA receptors. It has been reported that AMPA receptor is responsible for basal synaptic transmission whereas the NMDA receptor is responsible for regulating the efficacy of synaptic transmission. When Glu binds to AMPA, the membrane potential will change and Mg^{2+} will be expelled from the ion channel which coupling with NMDA receptor and Na^{+} and Ca^{2+} will be allowed to move in whereas K^{+} will be allowed to move out. The influx of Ca^{2+} then activates biochemical cascades that eventually strengthen the synapse. It is believed that some of these kinases bind directly to the C-terminus of the NR2B subunit, allowing efficient signal detection and amplification as shown in figure 2. The aforementioned processes are believed to be capable of supporting LTP for 1–3 h or the early phase of LTP. However, the maintenance of synaptic potentiation beyond the initial 3 h is associated with the function of protein kinase A (PKA) and ERK pathways (Chen *et al.*, 2005; Hayashi *et al.*, 2004). The time beyond this period is regarded as late-phase LTP and appears to require CREB (cAMP response element-binding) for the stimulate synaptogenesis and neurogenesis (Figure 2-3).

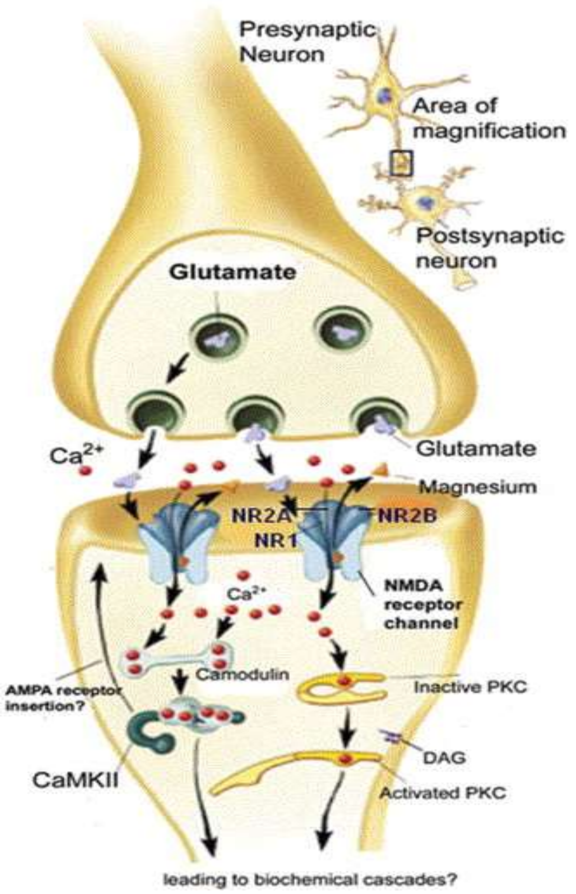

Figure 2-3 Schematic diagram illustrating syntactic plasticity or long term potentiation (LTP) (Wang *et al.*, 2006).

In addition to synaptic consolidation, the system consolidation also occurs. This process appears to be associated with the reorganization process in which memories from the hippocampal region where memories are first encoded are moved to the neo-cortex in a more permanent form of storage (Roediger *et al.,* 2007). When the new information is originally encoded and registered, memory of these new stimuli becomes retained in both the hippocampus and cortical regions (Frankland and Bontempi, 2005). Approximate 1 week after initial learning, memory is still retained in the hippocampus. Therefore, this period is regarded as hippocampus-dependent stage which is a period that hippocampus is 'teaching' the cortex more and more about the information and when the information is recalled it strengthens the cortico-cortical connection thus making the memory hippocampus-independent. Therefore, from one week and beyond the initial training experience, the memory is slowly transferred to the neo-cortex where it becomes permanently stored (Dudai, 2004). Recent imaging data show that hippocampus provides temporal and spatial context of memory consolidation, whereas the cortical traces are primarily context-free memory consolidation. Episodic memory, no matter whether new or old, relies on hippocampus-cortical networks while remote semantic memories can be retrieved independent of the hippocampus (Roediger *et al.*, 2007). At cellular level, it has been reported that the consolidating involves the transcription factor CREB (cAMP response element-binding) and BDNF (brain derived growth factor) (Lee *et al.*, 2004).

It is generally believed that long-lasting memory is stored in the form of structural synaptic modifications triggered by original learning. However, recent findings show that NMDA receptor and αCaMKII also play role in the memory storage in the critical storage (Wang *et al.*, 2006). Glutamate (Glu) appears to enhance both consolidation and storage whereas acetylcholine (ACh) hampers the consolidation but facilitates the storage (Micheau and Marighetto, 2010).

After being stored, memory can also be retrieved via retrieval process or a process of getting information out of memory**.** The retrieval process is associated with the activation of medial and lateral temporal lobes (MTL; hippocampus, parahippocampus), right frontal region (Nyberg *et al.*, 1996), medial and lateral prefrontal cortices (PFC), ventral parietal cortex, and posterior cingulate cortex (McDermott *et al.*, 2009). At the cellular level, it has been reported that a reduction in the postsynaptic settling (5-HT) receptor binding in the amygdala and periaquiductal gray matter appears to be a necessary component of memory retrieval processes of aversive memory. In addition, memory retrieval also involves a reduction of dopamine (DA) metabolism in the prefrontal cortex, hippocampus, and striatum (Molodtsova, 2008). Besides, serotonin and dopamine, acetylcholine can also hamper the memory retrieval retrieval (Micheau and Marighetto, 2010).

Memory can be classified according to various criteria. According to the temporal characteristic, memory can be divided in to 3 types as following;

1) Sensory or immediate memory is regarded as the routine ability to hold the ongoing experiences in mind for fractions of a second. The capacity of immediate memory is very large and each sensory modality appears to have its own memory register. A sensory memory exists for each sensory channel including *iconic memory* for visual stimuli, *echoic memory* for aural stimuli and *haptic memory* for touch. Information is passed from sensory memory into short-term memory by attention, thereby filtering the stimuli to only those which are of interest at a given time.

2) Short-term memory acts as a scratch-pad for temporary recall of the information under process. It decays rapidly within 200 ms and also has a limited capacity.

3) Long-term memory and entails the retention of information in a more permanent forms of storage for days, weeks, or even a lifetime.

Long-term memory can be divided into 2 types including explicit or declarative memory, the process involving the conscious, intentional recollection of previous experiences and information and implicit or non-declarative memory, the memory involving the performance of a task without conscious awareness of these previous experiences as shown in Figure 2-4 (Benfenati, 2007).

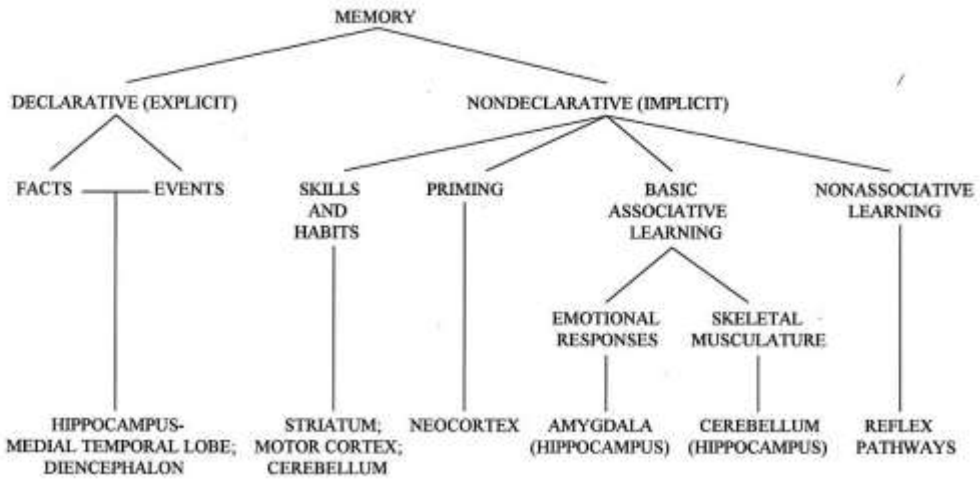

Figure 2-4 Various forms of learning and memory (Benfenati, 2007)

2.3 Memory Impairment Induced by Stress

Memory impairment is a symptom which a person experiences an abnormal level of forgetfulness and inability to recall past events in the life. The quality of memory is depending on numerous factors including the context which information is delivered, prior experiences, glycemic index, emotion and stress (Schwabe and Wolf, 2010).

The effects of stress on learning and memory are diverse and vary in their direction, strength, and occurrence. The variation in the types of responses is attribute in part to the organism properties, such as age, sex differences, and species. The responses are also dependent on the types of stressful events that occur, the length and intensity of those experiences, and finally, on the type of learning that is assessed (Shors, 2006).

Recent study has demonstrated that the modulation effect of stress on memory is associated with various memory phases including encoding, consolidation and retrieval (Schwabe *et al.*, 2010). Aforementioned processes are equally susceptible to disrupt by stress (Sandi, 2007) (Figure 2-5). Acute effect of glucocorticoid, the stress hormone, is reported to enhance memory consolidation (Roozendaal, 2002) but impairs retrieval of spatial–contextual information when it is administered to rats shortly before memory retention (Roozendaal *et al.*, 2004). However, it has been reported that the effect of glucocorticoid on consolidation depends on the emotional arousal state and the noradrenergic activation of basolateral complex of amygdala (BLA). The elevated catecholamine especially norepinephrine and epinephrine induced by stress will bind to the β-adrenoceptors on vagal afferents terminating in the nucleus of the solitary tract (NTS). Then, the noradrenergic cell groups in the NTS send the projection fiber either directly or indirectly to amygdala via locus coeruleus. Several lines of evidence also demonstrate that the direct administration of adrenoceptor agonists to amygdala enhances memory (McEwen and Sapolsky, 1995; Schwabe *et al.*, 2010). Therefore, amygdala is the area playing the important role on emotionally laden memories.

It has been found that acute effect of stress shows both positive and negative effects of memory whereas prolonged stress exposure shows only the negative effect on memory. The stress hormone, glucocorticoid, produces the significant dendritic atrophy and neuronal death in the hippocampus, the area containing abundant of glucocorticoid receptors (McEwen, 2000; You *et al.*, 2009). The possible underlying mechanism of neurodegeneration induced by glucocorticoid is associated with the exacerbation of excitotoxicity induced by glutamate (Jung-Man You *et al.*, 2009) such as the elevation of both extracellular calcium and the reactive oxygen species (Behl *et al.*, 1997).

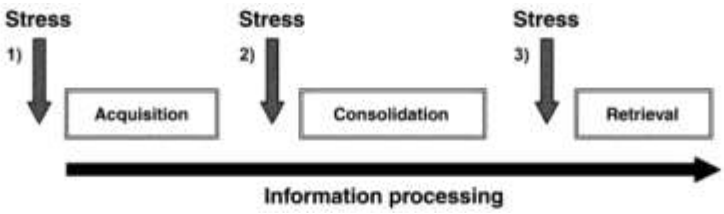

Figure 2-5 Information process related to memory function and disruption by stress (Sandi, 2007)

2.4 Neuro-endocrine Control of Sexual Response

Steroid hormones regulate male sexual behaviors primarily by genomically mediated influences on sexually relevant sensory processes, on neurotransmitter synthesis, release, and receptors, and on the responsiveness of appropriate motor effectors (Hull *et al.*, 2004). Male sexual behavior in all vertebrate species is dependent on Testosterone (T). Testosterone is secreted by the Interstitial Cell of Leydig cells and metabolized in target cells to estradiol (E2, by aromatization) or dihydrotestosterone (DHT, by 5α-reduction) (Hull and Dominguez, 2007). Both estradiol (E) and dihydrotestosterone (DHT) play an important role on the activation of mating, although E is more important for copulation and DHT for genital reflexes. In adult males, testosterone (T) deprivation after castration abolishes both sexual drive and copulatory behavior, and the declined sexual ability can be restored by T replacement (Tsai *et al.*, 2009). Accumulative lines of evidence point out that MPOA plays a crucial role in the regulation of male copulatory behavior in rats and many other mammals mammals (Tsai *et al.*, 2009). Dopamine in the medial preoptic area (MPOA) plays a significant role on the regulation of male copulation. Dopamine is released before and/or during copulation copulation (Hull *et al.*, 1997). It has been demonstrated that permanent bilateral lesions of the medial preoptic area and anterior hypothalamus (MPOA/AH) produce a drastic inhibition of male sexual behavior (Hurtazo *et al.*, 2008). One possible underlying mechanism of male sexual behavior induced by steroid hormones is mediated via the up-regulation of NOS in the MPOA. NO which is formed via NOS in turn increases both basal and female-stimulated dopamine release in the MPOA, which in turn enhances sexual motivation, genital reflexes, and copulatory motor patterns (Hull *et al.*, 2004).

2.4.1 Mechanism of penile erection

Penile erection is described as a neurovascular phenomenon which is under the influences of hormonal, biochemical and biomechanical factors both centrally and locally within the penis. The increased arterial inflow to the penis together with the deceased venous outflow gives rise to penile blood engorgement and penile erection. However this response is associated with many factors including corporeal smooth muscle relaxation, venoocclusive function and striated penile muscle activity (Wylie, 2008). The spinal cord contains all the necessary components for the coordination and initiation of penile erection. The tone of the penile vasculature and smooth muscles is controlled by both contractant and relaxant factors which are modulated by local and central processes. In addition, it has been found that normal erection also requires the participation of the sympathetic and somatic nervous systems which innervated penis (Geer and Janssen, 2000; Janssen *et al.*, 2007) (Figure 2.6).

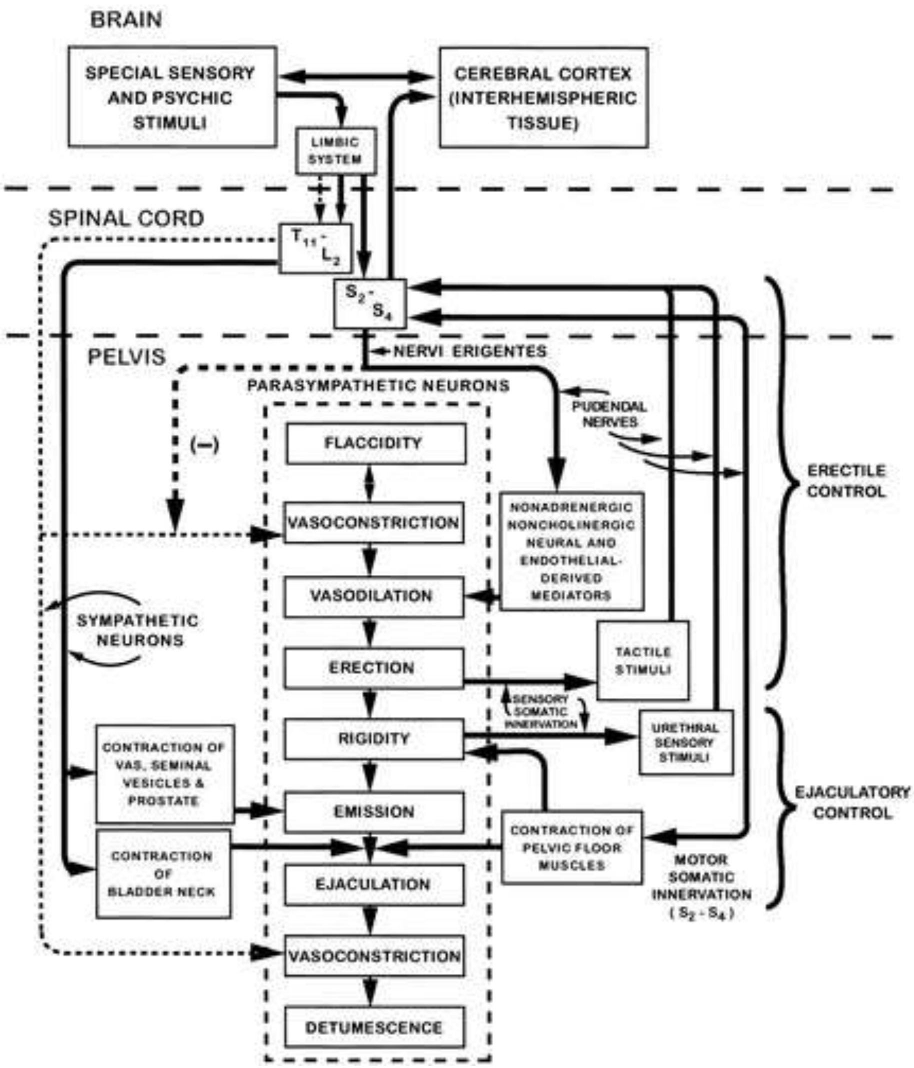

Figure 2-6 The interactions between autonomic and somatic innervations in the control of male sexual cycle (Kandeel *et al.*, 2001)

The parasympathetic pathway which supplies penis is originated in the intermediolateral aspect of the sacral cord (S2–S4), travels in the pelvic nerve and supply a vasodilating innervation to the corporeal bodies (Kandeel *et al.*, 2001), while the sympathetic pathway originates from the 11th segment of the spinal cord at thoracic level to the 2nd lumbar segment of spinal cord (Dean and Lue, 2005). Penile erection requires the inhibition of sympathetically controlled vasoconstriction in the penis (Geer and Janssen, 2000; Janssen *et al.*, 2007). While autonomic nervous system plays a crucial role on the erection mentioned earlier, the somatic nerves are primarily responsible for sensation and the contraction of the bulbocavernosus and ischiocavernosus muscles (Dean and Lue, 2005).

2.4.1.1 Local control of penile erection

The erection process may be divided into several phases as described following 1) The flaccid phase during which corporeal volume is small and corporeal pressure is low. 2) In the tumescence or filling stage the corporeal smooth muscle is relaxed due to reduction in the sympathetic tonus of the arteries allowing arterial inflow. The corporeal vessels are engorged with blood. This causes the corporeal pressure to rise and presses the relaxed veins against the tunica. The hemodynamic resistance of the veins increases significantly. 3) Rigidity is obtained due to contraction of the perineal muscles that generate high pressure peaks giving the rigidity required for full erection. 4) Detumescence begins with contraction of the corporal smooth muscles restricting arterial inflow and reopening venous return. The volume and pressure return to flaccid levels (Borowitz and Barnea, 2000)**.**

Acetylcholine, a neurotransmitter of the preganglionic parasympathetic neurons play an important role on the contractility of the corporeal smooth muscle fibers directly via the activation of cholinergic receptors on the endothelial cells (Figure 2-7). Nitric oxide (NO) has been identified in the corporeal tissue and is believed to be the endothelial-derived relaxation factors (Kandeel *et al.*, 2001). Nitric oxide (NO), a principle mediator of corporeal smooth muscle relaxation, is regarded as a nonadrenergic-noncholinergic (NANC) neurotransmitter of post-ganglionic parasympathetic nerve fibers which innervates a variety of smooth muscles including the penile corpus cavernosum (Toda *et al.*, 2005). NO produced by the sinusoidal endothelial cells and by the noncholinergic parasympathetic neurons diffuses into the adjacent smooth muscle cells and activates soluble guanylate cyclase to increase the intracellular cGMP concentration (Figure 2-8). The cGMP (second messenger molecule) appears to be the major intracellular effector of the smooth muscle cell relaxation via a biochemical cascade of protein kinases. A putative mechanism for cGMP induced corporeal smooth muscle relaxation involves protein kinase phosphorylation of myosin light chains directly or as a consequence of lowering intracellular calcium stores. cGMP levels are regulated by the enzyme phosphodiesterase (PDE), of which several subtypes exist. Drugs like sildenafil (Viagra), vardenafil (Levitra), and tadalafil (Cialis) exert their effect by blocking the degradation of cGMP through the inhibition of phosphodiesterase-5, which is relatively specific to the penis. This thus facilitates erection by increasing bioavailable cGMP in the penis (Janssen *et al.*, 2007). Phosphodiestrase inhibitors are emerging as an attractive physiological means for induction and/or prolongation of erection in man (Vickers and Satyanarayana, 2002).

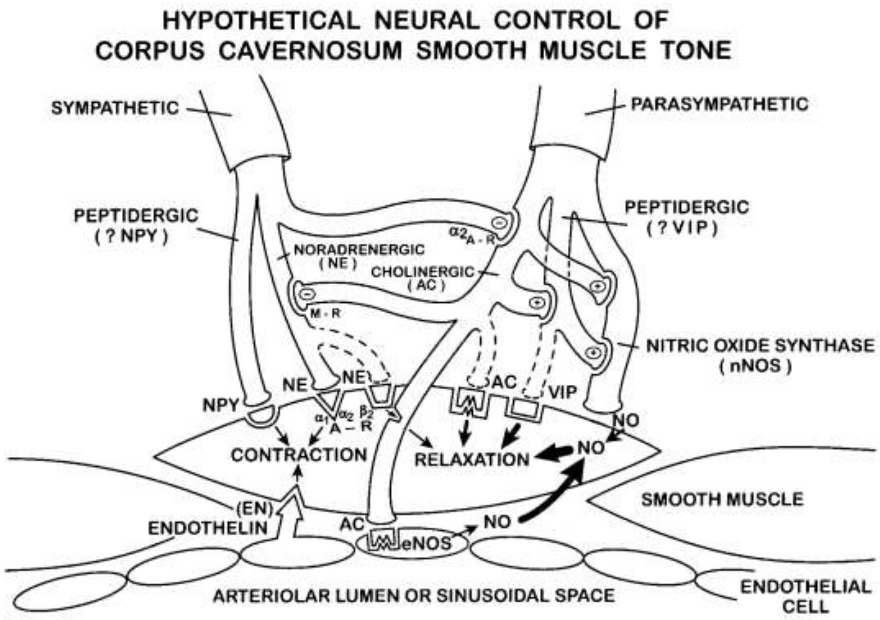

Figure 2-7 Neural control of the corporeal smooth muscle (Kandeel *et al.*, 2001).

Other noncholinergic parasympathetic neurotransmitters, include vasoactive intestinal polypeptide (VIP), bradykinin, peptide histidine methionine, pituitary adenylate cyclase-activating polypeptide, helospectin, galanin, calcitonin gene-related peptide (CGRP), and prostaglandin E-1can also promote smooth muscle relaxation, and hence the erectile response. The sympathetic innervation of smooth muscle cells includes norepinephrine (NE) and nonadrenergic (most likely neuropeptide Y fibers). α-1 and α-2 adrenoceptor activation, together with neuropeptide Y (NPY) and endothelin-1 (EN) actions, are responsible for smooth muscle cell contraction. Cross-talk between the two divisions of the autonomic innervation appears to exist, via an α-2 adrenoceptor and a muscarinic receptor on the parasympathetic and the sympathetic divisions, respectively (Kandeel *et al.*, 2001; Tanagho and McAninch, 2004).

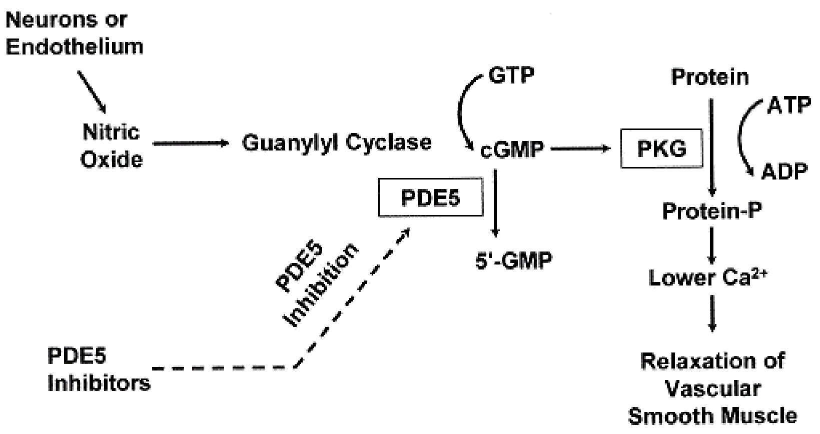

Figure 2-8 Nitric oxide–cyclic guanosine monophosphate (cGMP) signaling in cavernous smooth muscle. ADP adenosine diphosphate; ATP adenosine triphosphate; GTP guanosine triphosphate; PDE phosphodiesterase; PKG protein kinase G (Rosen and Kostis, 2003)

2.4.1.2 Supraspinal control of erection

Brain areas that are relevant to penile erection include the amygdala, hippocampus, locus coerulus, periaqueductal gray, and hypothalamus. Medial preoptic area (MPOA) is believed to have a function on the processing of sensory stimuli whereas the paraventricular nucleus (PVN) is believed to exert the role on the integration of genital and nongenital autonomic processes (Janssen *et al.*, 2007). The neurons in these hypothalamic nuclei contain peptidergic neurotransmitters, including oxytocin and vasopressin, which may be involved in penile erection (Meisel and Sachs, 1994). Several centers in brain stem and medulla are also involved in sexual function. The catecholamine cell group and locus ceruleus have been shown to provide adrenergic innervation to hypothalamus, thalamus, neocortex and spinal cord. Projections from the nucleus paragigantocellularis, which provides the inhibitory serotonergic innervation, have also been demonstrated in hypothalamus, the limbic system, the neocortex and the spinal cord (Dean and Lue, 2005). Serotonin can also have facilitory effects, depending on receptor location and subtype, and this may be relevant to the sometimes conflicting findings on sexual effects of serotonin agonists and antagonists (Janssen *et al.*, 2007).

2.4.2 Phases of sexual response

Sexual stimulation of the human male results in a series of psychological, neuronal, vascular, and local genital changes. At least three different classifications for these changes have been described. Psychosexual response cycle consists of four phases: excitement, plateau, orgasm, and resolution (Kandeel *et al.*, 2001; Kolodny *et al.*, 1974). In addition, sexual cycle can also be divides into two interrelated events as excitement into latency and tumescence; plateau into erection and rigidity; orgasm into emission and ejaculation; and resolution into detumescence and refractoriness (Kandeel *et al.*, 2001). The third classification focuses on the functional activities during the sexual cycle by adding an initial phase of desire or libido. Thus, the normal male sexual response cycle can be functionally divided into five interrelated events that occur in a defined sequence: libido, erection, ejaculation, orgasm, and detumescence (Yakubu *et al.*, 2007).

2.4.2.1 Libido or sexual desire

Libido is defined as the biological need for sexual activity (the sex drive). It is frequently expressed as sex-seeking behavior. Its intensity is variable between individuals as well as within an individual over a given time. Higher serum testosterone appears to be associated with greater sexual activity in healthy older but not younger (Yakubu *et al.*, 2007).

2.4.2.2 Erection

Erection is the enlarged and rigid state of the sexually aroused penis sufficient enough for vaginal penetration. It results from multiple psychogenic and sensory stimuli arising from imaginative, visual, auditory, olfactory, gustatory, tactile, and genital reflexogenic sources (Yakubu *et al.*, 2007).

2.4.2.3 Ejaculation

Ejaculation is the act of ejecting semen. The ejaculation phase is controlled by sympathetic innervation of the genital organs and occurs as a result of a spinal cord reflex arc (Kandeel *et al.*, 2001). It is made up of two sequential processes. The first process called emission is associated with deposition of seminal fluid into the posterior urethra while the second process is the true ejaculation, which is the expulsion of the seminal fluid from the posterior urethra through the penile meatus (Yakubu *et al.,* 2007).

2.4.2.4 Orgasm

This is the climax of sexual excitement. The entire period of emission and ejaculation is known as the male orgasm (Yakubu *et al.*, 2007).

2.4.2.5 Detumescence

This phase is the phase which penis returns to the flaccid state. Vasoconstriction of the arterioles and reversal of events within the contractile corporeal units divert the blood away from the cavernous sinuses and allow an increase in the venous drainage of their contents (Kandeel *et al.*, 2001).

2.4.3 Assessment of sexual function

Sexuality is a complex, multi-dimensional phenomenon that incorporates biological, psychological, interpersonal and behavioral dimensions. Sexual behavior in male rats consists of three distinct phases:

2.4.3.1 Mount

Mounting is defined as the climbing of one animal by another usually from the posterior end with the intention of introducing one organ into another may also be operationally defined as the male assuming the copulatory position, but does not insert its copulatory organ (the penis) into the vagina (Agmo, 1997).

2.4.3.2 Intromission (vaginal penetration)

Introduction of one organ or parts into another e.g. the penis into the vagina. This behavior starts with a mount, but suddenly the male makes a deep thrust forward and stops pelvic thrusting. He then vigorously withdraws and always licks his genitals. A male will never mount again immediately after an intromission (Agmo, 1997).

2.4.3.3 Ejaculation

During this process, semen is expelled. This behavior starts with an intromission, but after vaginal penetration the male remains on the female for 1–3 s. Rhythmic contractions of the posterior abdomen are clearly visible. Male then slowly raise with his forelegs held open. At ejaculation, it is the female that moves away from the male. Male then licks his genitals and remains inactive for several minute (Agmo, 1997; Yakubu *et al.*, 2007).

2.4.4 Some standard parameters of sexual behavior

2.4.4.1 Mount latency: Time from the introduction of the female until the first mount. Incomplete mounts without pelvic thrusting or badly oriented mounts are disregarded (Agmo, 1997).

2.4.4.2 Intromission latency: Vaginal penetration, time from introduction of the female until the first intromissions.

2.4.4.3 Ejaculation latency: Time from the first intromission until the first ejaculation.

2.4.4.4 Mount frequency: The number of mounts without intromission within 30 minute.

2.4.4.5 Intromission frequency: The number of intromissions within 30 minute.

2.4.4.6 Ejaculation frequency: The number of ejaculation within 30 minute.

2.4.5 Sexual dysfunction

Sexual dysfunction in men refers to repeated inability to achieve normal sexual intercourse. It can also be viewed as disorders that interfere with sexual response cycle. These disorders make it difficult for a person to satisfy or to have sexual intercourse. Sexual dysfunction can be classified as disorders of desire, disorders of orgasm, erectile dysfunction, disorders of ejaculation and failure of detumescence. 1) Disorders of desire can involve either a deficient or compulsive desire for sexual activity. It can occur during the desire phase including hypoactive sexual desire and compulsive sexual behaviors. 2) Erectile dysfunction (ED) is a problem with sexual arousal, can be defined as the difficulty in achieving or maintaining an erection sufficient for sexual activity or penetration. 3) Disorders of ejaculation: There exists a spectrum of disorders of ejaculation ranging from mild premature to severely retard or absent ejaculation including premature ejaculation which is the most common male sexual dysfunction, painful ejaculation, inhibited or retarded ejaculation and retrograde ejaculation. 4) Disorders of orgasm is defined as a persistent or recurrent delay in, or absence of orgasm after a normal sexual excitement phase during sexual activity. 5) Failure of detumescence is a prolonged erection usually lasting for between 4 h or greater (Yakubu *et al.*, 2007).

Male sexual dysfunction can occur as the result of various factors including psychological disorders such as stress, anxiety and depression. Moreover, some diseases and disorders could also induce of sexual dysfunction such as low level of testosterone, diabetes, hypertension, penile disease (Peyronie's disease), neurological disorders (Parkinson's disease, stroke, Alzhemier's disease, spinal cord and nerve injury), side effects of some drugs, chronic alcohol consumption, smoking and aging (Kandeel *et al.*, 2001; Mechanick *et al.*, 2008).

2.4.6 Phosphodiesterase-5 Drugs and therapy of erectile dysfunction

Phosphodiesterases (PDEs) catalyze the hydrolysis of the second messengers cAMP and cGMP that are responsible for activating a pathway of signaling events leading to smooth muscle relaxation. Both second messengers mentioned earlier are involved in signal pathways within the corpus cavernosum. Selective phosphodiesterase type 5 inhibitor (PDE-5-I), Sildenafil, increased cyclic GMP leading to the amplification of the endogenous NO-cGMP signaling pathway (Cirino *et al.*, 2006) in corpus cavernosum resulting in the relaxation of human corpus cavernosum.

2.5 Stress and Sexual Dysfunction

It is believed that various forms of stress, especially psychological stress, cause a decrease in sexual function. Reproductive activity is one of the main functions that becomes altered and inactivates during the adaptive response to stress. Males under stress may exhibit suppression of testosterone secretion, spermatogenesis and libido (Johnson *et al.*, 1992; Pack and Palkovits, 2001). The chronic exposure to stressors increases HPA axis activity and concomitantly reduces Hypothalamus-Pituitary-Gonadal (HPG) axis activity, attention was focused on the role of glucocorticoids in reproductive dysfunction induced by stress (Figure 2-9) (Retana-Marquez *et al.*, 2003).

There are two main sources of ROS in semen: leukocytes and immature spermatozoa. Of these, leukocytes are considered to be the primary source. Leukocytes, particularly neutrophils and macrophages, are associated with excessive

ROS production that ultimately leads to sperm dysfunction (Agarwal and Prabakaran, 2005). The two main sites of ROS production are the mitochondrion and the sperm plasma membrane. Spermatozoa are sensitive to oxidative stress because they lack cytoplasmic defenses (Agarwal *et al.*, 2005; Gil-Guzman *et al.*, 2001). Moreover, the sperm plasma membrane contains lipids in the form of polyunsaturated fatty acids, which are vulnerable to attack by ROS (Agarwal *et al.*, 2005). Increased production of ROS (superoxide and peroxynitrite) reduces the effective NO concentration available for cavernosal muscle relaxation (Agarwal *et al.*, 2006).

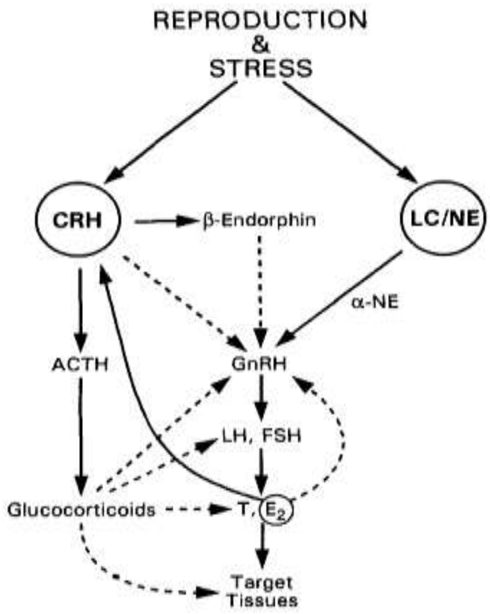

Figure 2-9 A schematic representation of the interactions between the stress system and the reproductive axis. Solid lines represent direct or indirect activation, and dashed lines, direct or indirect inhibition (Stratakis and Chrousos, 1995)

2.6 Oxidative Stress and Stress

When the regulation of oxidation stress is disturbed, excess oxidation stress is observed (Ma, 2010). The elevation of oxidation stress concentrations can induce cell death, apoptosis and senescence (Mahadik and Mukherjee, 1996; Ziech *et al.*, 2010).

2.6.1 Reactive oxygen species (ROS) and free radical

Free radical species are unstable unpaired electron and highly reactive. They become stable by acquiring electrons from the others. There are two major types of free radical species: reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Agarwal *et al.*, 2005; Machlin and Bendich, 1987). Free radicals can originate endogenously from normal metabolic reactions or exogenously as components of tobacco smoke and air pollutants and indirectly through the metabolism of certain solvents, drugs, and pesticides as well as through exposure to radiation radiation (Machlin and Bendich, 1987). Oxidative stress is caused by exposure to reactive oxygen intermediates, such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$) which can damage polyunsaturated fatty acids in cellular membranes, nucleotides in DNA, and critical sulfhydryl bonds in proteins (Machlin and Bendich, 1987; Storz and Imlay, 1999).

2.6.1.1 Sources of reactive oxygen species (ROS)

ROS can be generated in multiple compartments and by multiple enzymes in cells (Figure 2-10) (Ma, 2010). Mitochondria are considered to be the main cellular source of superoxide (Bartosz, 2009). Oxidative phosphorylation in mitochondria uses controlled oxidation of NADH or $FADH_2$ to generate a potential energy for protons across the mitochondrial inner membrane. This potential energy is then used to phosphorylate ADP by the F_1–F_0 ATPase. Along the respiratory chain, electrons derived from NADH or $FADH_2$ can directly react with oxygen and generate free radicals. Production of superoxide radicals in mitochondria occurs primarily in complex I (NADH dehydrogenase) and complex III (ubiquinonecytochrome c reductase) (Ma, 2010). Cytoplasmic enzymes contribute to oxidative stress, including the expanding family of ROS-generating NADPH oxidases (NOX), The NOX enzymes share the capacity to transport electrons across the plasma membrane and to generate superoxide and other downstream ROS. Additional sources of cytoplasmic ROS production include cytochrome P450s, lipoxygenases, and one-electron reduction of quinones by NADPH:cytochrome P450 reductase (Ma, 2010).

Toxic metals can induce the production of ROS by directly acting as catalytic centers for redox reactions with molecular oxygen or other endogenous oxidants, or by promoting the iron dependent Fenton reaction.

Superoxide is also formed by autoxidation of flavins, hydroquinones, thiols and metals (free or protein-bound).Moreover, ROS are formed by interaction of light, UV and ionizing radiation and ultrasound with biologic material (Bartosz, 2009).

There are four main pathways account for ROS production especially in the critically ill: 1) The mitochondrial respiratory chain produces $O_2^{\bullet -}$ as a by-product of the reaction of molecular oxygen with semi-ubiquinone. 2) The NADPH oxidase enzyme of neutrophils and macrophages is activated in cell stimulation and can produce massive amounts of $O_2^{\bullet -}$ as a microbiocidal mechanism. This pathway is probably predominant in the overproduction of ROS during severe sepsis. 3) The ubiquitous xanthine oxidase enzyme is activated during ischemia, and produces massive amounts of $O_2^{\bullet -}$ during the reperfusion phase. This pathway is probably activated during major cardiac and vascular surgery and during the transplantation of solid organs. 4) Some metallic ions (iron, copper) are released during cell destruction/lysis and can amplify the oxidative stress, as cofactors of the conversion of hydrogen peroxide into hydroxyl (Berger, 2005).

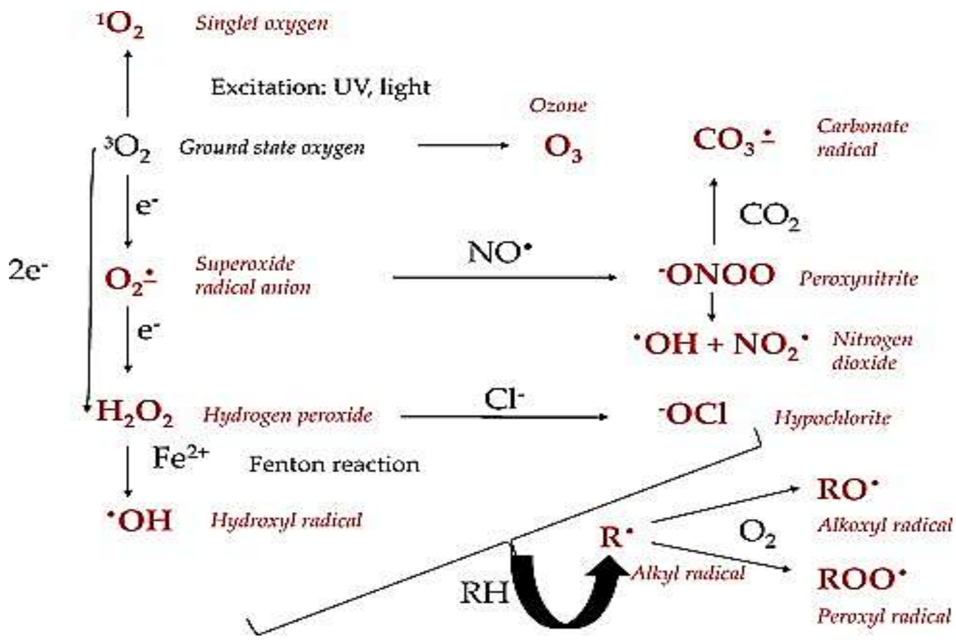

Figure 2-10 Main reactive oxygen species; RH; organic molecule (Bartosz, 2009)

2.6.1.2 Antioxidants

To defenses the damaging effects of ROS, aerobic organisms involved both nonenzymatic and enzymatic antioxidant defenses (Figure 2-11).

The antioxidant defense system is perhaps the major mechanism by which cells counteract ROS production. The system includes enzymatic scavengers (Figure 2-12), such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHpx). Two SOD enzymes exist in the cell: SOD_1 (Cu/ZnSOD) is a copper- and zinc-containing enzyme primarily localized in the cytoplasm and SOD_2 (MnSOD) is a manganese-dependent enzyme in the mitochondrial matrix. SOD catalyzes the conversion of superoxide anions to hydrogen peroxide, whereas catalase and glutathione peroxidase convert hydrogen peroxide to water. Recently, a new family of peroxide scavengers termed peroxiredoxins was identified. Peroxiredoxins reduce peroxides in the presence of thioredoxins. Myeloperoxidase is found in the granules of neutrophils and catalyzes the conversion of H_2O_2 and Cl^- to more reactive hypochlorous acid (OCl^-), which is important for the bactericidal activity of neutrophils (Ma, 2010). Under some conditions CAT can act as an efficient peroxidase. SODs deal with the first product of the univalent reduction of O_2, converting it to H_2O_2, which must then be destroyed by CAT and/or peroxidases. Thus, the SOD and CAT serve as antioxidant defenses (de Zwart *et al.*, 1999; Scandalios, 2005).

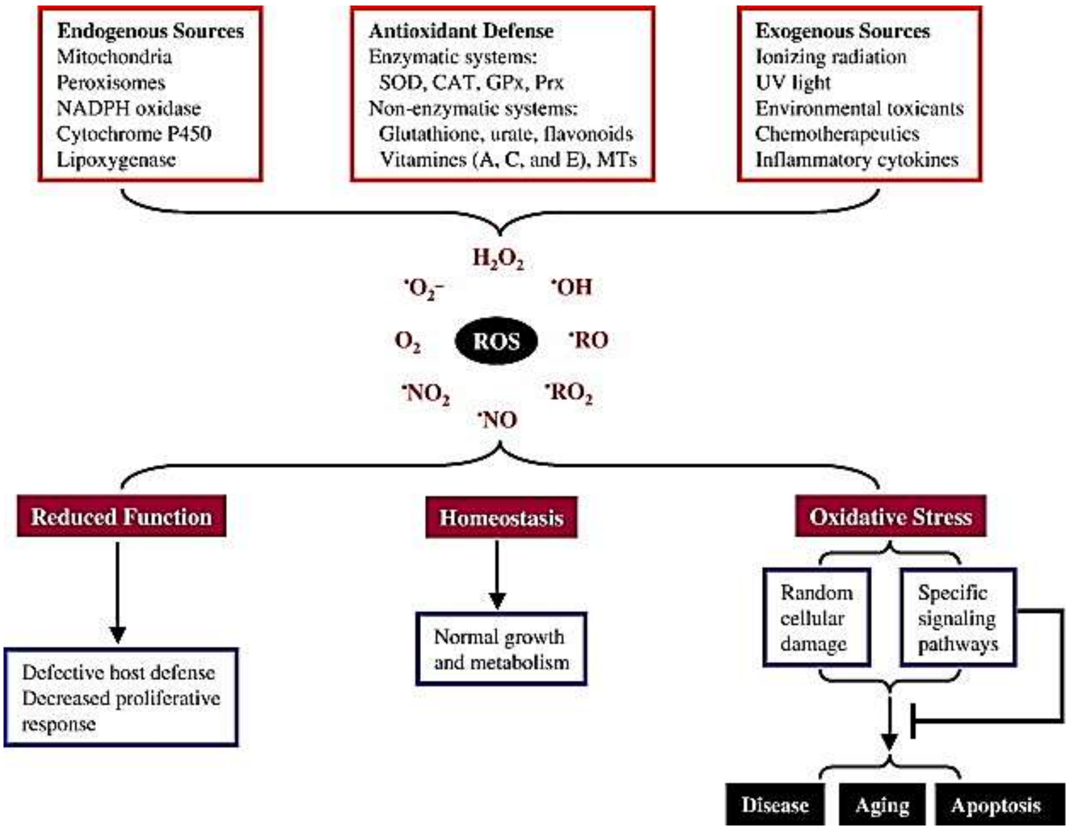

Figure 2-11 Antioxidant defenses, metabolism and biological effects of reactive oxygen species (ROS) (Ma, 2010)

Non-enzymatic defenses are important in scavenging ROS; these include glutathione, vitamins (C and E), pyruvate, flavonoids, carotinoids, urate, and many plant-derived antioxidants. Glutathione is likely the most important antioxidant of low molecular mass, because it is present in millimolar concentrations in cells. Metallothioneins (MT) I and II are small proteins rich in cysteine thiols. MTs are highly inducible by metals and oxidants and thus, are critical in protection against exogenous oxidants, such as the carcinogenic metals cadmium and arsenic (Ma, 2010).

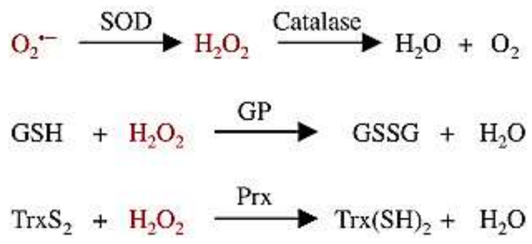

Figure 2-12 Enzymatic elimination of ROS. SOD, superoxide dismutase; Prx, peroxyredoxin; GP, glutathione peroxidase (Ma, 2010)

Several dietary micronutrients contribute greatly to the protective system. The level of dietary intake of all the antioxidant micronutrients directly affects the circulating level of these nutrients and the activity of the antioxidant metalloenzymes. Thus, low intakes of one or more of these antioxidant nutrients could reduce the body's defenses against free radical damage and increase susceptibility to health problems associated with free radical damage (Machlin and Bendich, 1987).

2.6.2 Reactive oxygen species in pathophysiological processes

Reactive oxygen species (ROS) play a key role in the pathogenesis of chronic degenerative diseases including cancer, autoimmune, inflammatory, cardiovascular and neurodegenerative diseases and aging process (Koleckar *et al.*, 2007; Machlin and Bendich, 1987). ROS may induce DNA mutations, protein inactivation, lipid peroxidation, cell apoptosis or abnormal proliferation, eliciting the occurrence of diseases from the cellular and molecular levels (Gan *et al.*, 2002).

2.7 Selected Thai medicinal plants

2.7.1 *Moringa oleifera* Lam (Family: Moringaceae)

Drumstick tree, known as horseradish tree in English, It is reported to contain alkaloids, flavonoids, anthocyanins and proanthocyanidins. It possesses anti-inflammatory, antioxidant, antimicrobial, antihyperlipidaemic, antifertility, anticancer, antihepatotoxic and antiulcer activities (Schmidt and Mwaura, 2010). It has long been used in nutritional, industrial and medical fields. *M. oleifera* leaves are used for treating various ailments including constipation, headache, fever, and diabetes

(Makonnen *et al.*, 1997). In addition, it has been demonstrated that *M.oleifera* leaf extracts inhibit 6-beta-hydroxylation of testosterone (Monera *et al.*, 2008). *M.oleifera* leaf powder improved sperm count, histology of testis and epididymis of hyperglycaemic mice (Priyadarshani and Varma, 2014). Acute toxicity of aqueous leaf extract of *M.oleifera* was estimated to be 1585 mg/kg (Awodele *et al.*, 2012).

Figure 2-13 *Moringa oleifera* Lam

2.7.2 *Anacardium occidentale* Linn (Family Anacardiaceae)

A.occidentale is a native plant to the dry areas of the Central America and northern part of South America. Now, it is widely distributed in Thailand. *A.occidentale* leaves have been used as the traditional medicine to treat diabetes, diarrhea and impotence including enhancing aphrodisiac. *A.occidentale* leaves contain high level of flavonoids, mainly glycosides of quercetin and kaempferol, and hydroxybenzoic acid. It has been reported that flavonoids and derivatives (quercetin and kaempferol) act like non-selective PDE inhibitors (da Silva *et al.*, 2012).

It was found that, *A. occidentale* hydroethanolic leaves extract showed antiulcerogenic effect (Konan and Bacchi, 2007) and did not produce acute toxicity in rats in doses up to 2000 mg/kg (Konan *et al.*, 2007). The hexane extract of *A. occidentale* leaves showed the improvement of sexual performance (Chauhan *et al.*, 2014) and no acute toxicity in mice after the administration at doses less than 6,000 mg/kg BW (Tédong *et al.*, 2008). *A. occidentale* aqueous extract inhibited the hyperglycemic action of streptozotocin-induced diabetes in rat (Kamtchouing *et al.*, 1998).

Figure 2-14 *Anacardium occidentale* Linn.

2.7.3 *Nelumbo nucifera* Gaertn (Family*:* Nelumbonaceae)

Nelumbo nucifera or sacred lotus is an aquatic herb with creeping rootstocks (Mukherjee *et al.*, 1996). All parts of *N. nucifera* are used as medicine. Lotus alkaloids dilate the blood vessels and reduce the blood pressure (Sridhar and Bhat, 2007). In addition, flowers of *N.nucifera* have been traditionally used to treat diarrhea, cholera, fever, hepatopathy and many bleeding disorders. Moreover, it is used for treating premature ejaculation, abdominal cramps and bloody discharges (Mukherjee *et al.*, 2009). Hydro alcoholic extract of *N. nucifera* flowers seems to have potential value for the treatment of diabetes (P. Manimekalai, 2012). Moreover, flower extract of *N.nucifera* were used for aphrodisiac activity and increased testosterone level (BI *et al.*, 2012). Acute toxicity test, 95% ethanolic extract of *N.nucifera* at dose of 2,000 mg/kg BW could not produce any signs or symptoms of toxicity and mortal rat was not found during the period of observation (Sakuljaitrong *et al.*, 2013).

Figure 2.15 *Nelumbo nucifera* Gaertn.