Role of Tryptophan, Curcumin and Cocoa in Experimental Model of Depression in Rats

A Thesis
Submitted To Faculty of Science, Ain Shams University for Partial Fulfillment of Master Degree of Science in Biochemistry

By
Eman Nasr Mohammed Hosny
(B.Sc. Biochemistry, 2003)

Under Supervision of

Prof. Dr. Amani F.H.Nour Eldeen
Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

Prof. Dr. Karima Abbas EL-Shamy
Professor of Medical Physiology
Medical Physiology Department
Medical Research Division
National Research Centre

Dr. Azza Salah Helmy
Assistant Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

(2011)

ABSTRACT

The present study aimed to estimate the anti-depressant effect of curcumin and cocoa powder on experimentally induced animal model of depression (depression was induced by injection of reserpine) with comparison with well-established antidepressant drugs; fluoxetine and tryptophan. Also, the study evaluated the safety effect of curcumin and cocoa powder on liver and kidney functions. The study included 200 male albino rats, divided into two main groups: Group I (GI): control group: included 100 rats and were subdivided equally into 5 subgroups: normal control, tryptophan, fluoxetine, curcumin, and cocoa powder subgroups. Group II (GII): depressed group: included 100 rats and were i.p. injected with reserpine for 14 days to establish the animal model of depression. Starting from the 15th day of daily reserpine injection, animals were divided equally into the following 5 sub-groups: reserpinized animals (without treatment), tryptophan treated, fluoxetine treated, curcumin treated and cocoa powder treated subgroups. After 14 and 30 days of daily treatment with the previous treatments, the following studies were made: behavioral studies (immobility time was measured to evaluate the antidepressant effect of different treatments), biochemical studies (serum alanine transaminase, aspartate transaminase activities, serum urea and creatinine levels were measured to evaluate the safety of different treatments on both liver and kidney) and neurochemical studies (serotonin "5-HT", norepinephrine "NE" and dopamine "DA" were measured in cortex and hippocampus brain areas). Results indicated that increased immobility time induced by reserpine was restored into normal control levels by tryptophan, curcumin and cocoa powder treatment for 14 and 30 days and by fluoxetine treatment for 30 days. The daily treatment with reserpine, tryptophan, fluoxetine, curcumin and cocoa powder had safe effect on liver function. The present data demonstrated the safety of tryptophan, fluoxetine, curcumin and cocoa powder on liver function. The daily treatment with curcumin and cocoa powder had the ability to restore the damage induced by reserpine on kidney function. The daily injection of reserpine resulted in significant decrease in cortical and hippocampal monoamine levels. The daily treatment of reserpinized rats with tryptophan for 14 and 30 days restored the significant decrease in cortical 5-HT and DA induced by reserpine to normal control levels but failed to restore the significant decrease in cortical NE and hippocampal monoamine levels. This may explain the reduced efficacy of tryptophan alone as an antidepressant and may be used as a co-drug

with other antidepressant drugs. When fluoxetine treatment was applied to reserpinized animal model of depression, the significant decrease in cortical and hippocampal monoamines induced by reserpine persisted after 14 days of daily fluoxetine treatment. Daily treatment of reserpinized rats with fluoxetine for 30 days returned the significant decrease in cortical 5-HT, DA and hippocampal 5-HT levels to normal control values. The daily treatment of reserpinized rats with curcumin and cocoa powder for 14 and 30 days changed the significant decrease in cortical monoamines to control levels. The treatment of reserpinized rats with curcumin and cocoa powder for 14 days returned the significant decrease of hippocampal NE and DA to normal control values. Furthermore, the daily treatment of reserpinized rats with curcumin and cocoa powder for 30 days restored the significant decrease in hippocampal monoamines levels to normal values. It could be concluded that curcumin and cocoa powder can be used safely and efficiently alone as antidepressants.

CONTENTS

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Part I: Depression

A. Epidemiologic characteristics:

Depression is a highly debilitating disorder that has been estimated to affect up to 21% of the world population **(Schechter *et al.,* 2005)**. Depressive illnesses carry significant risks of death and disability. About 15% of patients with a mood disorder die by their own hand, and at least 66% of all suicides are preceded by depression **(Bostwick and Pankratz, 2000)**. Despite the fact that depression is a psychiatric disorder; the World Health Organization predicts that it will be the second leading cause of death by the year 2020 due to complications arising from stress and the cardiovascular system **(Schechter *et al.,* 2005)**. Depressive disorders are associated with poor work productivity, as indicated by a 3-fold increase in the number of sick days in the month preceding the illness for workers with a depressive illness compared with coworkers who did not have such an illness **(Parikh *et al.,* 1996 & Kessler *et al.,* 1999)**. Depressive illnesses also affect family members

and caregivers **(Denihan, *et al.,* 1998)**, and there is increasing evidence that children of women with depression have increased rates of problems in school and with behavior, and have lower levels of social competence and self-esteem than their classmates with mothers who do not have depression **(Goodman and Gotlib, 1999)**.

Cooper and Magnus (1984) have categorized depressive reactions into three types: reactive (e.g., neurotic depression, grief and depression associated with a personality disorder), symptomatic (e.g., depression following the use of drugs such as reserpine or α-methyldopa, or secondary to organic or other psychiatric disorders) and endogenous (e.g., the depression of unipolar and bipolar disorders). Depression interspersed with periods of mania is termed "bipolar" while that seen in the absence of manic symptoms is termed "unipolar" **(Elliott and Stephenson, 1989)**.

B. Etiology of depression:

It has become clear that depression is a very complex condition that involves abnormalities of the sympathetic nervous system as well as the endocrine and immune systems **(Szelenyi and Selmeczy, 2002)**. However, neurobiological basic research as well as clinical studies have revealed that

monoamines (5-HT, NE and DA) have a crucial role in the development of the depression syndrome. The view that 5-HT has multiple functional roles in depression is supported by clinical and experimental evidence suggesting that 5-HT is involved in the regulation of mood, sleep, memory, learning and sexual behavior, all of which are deranged to varying extents in patients with severe depression **(Naughton *et al.*, 2000)**. Depression is characterized most often by anhedonia or the loss of interest or pleasure in normal daily activities and feelings of sadness. Additional symptoms may include sleep disturbances, a gain or loss of weight accompanied, respectively, by increase or decrease in appetite, recurrent inappropriate feelings of guilt, psychomotor agitation, difficulty concentrating and thinking including indecisiveness and thoughts of death or suicide **(Schechter *et al.*, 2005)**.

The chemical underpinnings of depression for the last 50 years have been referred to as the monoamine hypothesis that postulates that the debilitating and often chronic symptoms of depression result from perturbations in 5-HT, NE and/or DA transmission. This hypothesis spawns from work done in the late 1950s showing that monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants

(TCAs), which elevate levels of monoamines by preventing their metabolism and blocking their reuptake, respectively, were effective antidepressants **(Stahl, 1998a; Eriksson, 2000 & Millan, 2004)**. Interestingly, further support for the chemical hypothesis of depression is based on clinical data where the side effects of reserpine as an antihypertensive agent in the 1960s suggested that depleting brain monoamines had detrimental effects on mood **(Goodwin and Bunney, 1971)**. The majority of subsequent hypotheses suggest that depression arises from the dysregulation of one or more neurotransmitters or neuroregulators in areas of the brain involved in mood regulation, e.g., the cerebral cortex and limbic system **(Anand and Charney, 2000 & Ressler and Nemeroff, 2000)**. The hippocampus is an area of the limbic system of the brain that is involved in emotion and memory **(Heales, 2005)**.

The hippocampus and the cerebral cortex are implicated in the pathology of major depression, stress responses and/or actions of antidepressant treatment. Recent imaging studies in humans revealed that the hippocampus underwent selective volume reduction in stress-related neuropsychiatric disorders such as recurrent depressive illness **(Bremner *et al.*, 2000 & Sapolsky, 2000)**. In adult

rats, chronic antidepressant treatment increased neurogenesis in the hippocampus **(Malberg *et al.*, 2000)**, and upregulated the cAMP signaling pathway-mediated gene transcription in the cortex and the hippocampus **(Thome *et al.*, 2000)**. These studies clear the relation of cortex and hippocampus with depression.

Chronic stress is a risk factor for the onset of major depression. Chronic unpredictable stress is often used as an animal model of depression in rodents because it induces symptoms of depression, including increased corticosterone levels and impaired learning and memory abilities **(Gold and Chrousos, 2002; Warner-Schmidt and Duman, 2006 & Xu *et al.*, 2006)**.

Preclinical studies have suggested that stress may alter brain structure and activity **(Kim and Diamond, 2002 & Rosenbrock *et al.*, 2005)**. The stress sensitive hippocampus is one region that has recently received significant attention in mood disorder research. Chronic stress and consequent elevated glucocorticoid exposure are associated with structural and functional changes in the hippocampus, including atrophy of apical dendrites of CA3 pyramidal neurons **(Campbell and MacQueen, 2004 & Li *et al.*, 2004)**. More importantly, chronic unpredictable stress

decreases neurogenesis in the adult hippocampus, which may contribute to this hippocampal atrophy **(Joels *et al.*, 2004; Beauquis *et al.*, 2006 & Li *et al.*, 2006)**.

Part II:Monoamine neurotransmitters

Nerve cells communicate with each other and with target tissues by secreting chemical messengers, called neurotransmitters **(Nath, 1996 & Heales, 2005)**. Traditionally, for a molecule to be labeled as a neurotransmitter a number of criteria have to be met. These include: the synthesis of the molecule must occur within the neuron (i.e. all biosynthetic enzymes, substrates, cofactors, etc., are present for *de novo* synthesis), the storage of the molecule occurs within the nerve ending prior to release (e.g. in synaptic vesicles), the release of the molecule from the presynaptic ending occurs in response to an appropriate stimulus such as action potential, there is binding and recognition of the putative neurotransmitter molecule on the postsynaptic target cell and the mechanisms exist for the inactivation and termination of the biological activity of the neurotransmitter **(Heales, 2005)**.

5-HT, NE, and DA are widely distributed neurotransmitter systems in the mammalian central nervous system, regulating a considerable array of behaviors including mood, appetite, cognition, libido, anxiety, and aggression **(Nemeroff, 2002)**. All three monoamines are important in the regulation of mood, emotion, and cognitive function and many of these functions have been demonstrated to be impaired in patients with depression **(Nemeroff, 2002)**.

NE, epinephrine and DA are also known as catecholamines. These monoamines share common pathways in their synthesis, where they are synthesized from the same precursor (tyrosine) which is converted inside the nerve terminal by tyrosine hydroxylase (the rate-limiting step enzyme) to 3,4 dihydroxyphenylalanine (DOPA). DOPA is then converted by DOPA decarboxylase to DA, which is converted to NE by dopamine-β-hydroxylase **(Iverson, 1991; Granner, 1999 & Meijer *et al.*, 2003)**. The other monoamine serotonin is also known as 5-hydroxytryptamine (5-HT). 5-HT is synthesized from tryptophan, which is converted inside the nerve terminal to 5-hydroxytryptophan by tryptophan hydroxylase (which is rate-limiting step) then

to 5-HT by the action of aromatic amino acid decarboxylase **(Fuller, 1980; Meijer *et al.*, 2003 & Heales, 2005)**.

All monoamines after their synthesis are concentrated in vesicles at the nerve terminal by a specific vesicular monoamine transporter **(Njus *et al.*, 1986)**.When the action potential reaches the monoamine nerve terminals, it causes the vesicle fusion with the presynaptic membrane and the release of the monoamine into the synaptic cleft in a process known as exocytosis **(Llinas, 1977 & Vizi, 2000).**

The released monoamine will act on specific receptors located either on postsynaptic or presynaptic membranes. Stimulation of the postsynaptic receptors results in changes in the properties of the postsynaptic membrane with either a shift in membrane potential when the receptors are coupled to ion channels (known as ionotropic receptors), or biochemical changes in intracellular cyclic nucleotides, protein kinase activity, and related substrate proteins when the receptors are coupled to G-proteins (known as metabotropic receptors) **(Starke *et al.*, 1977)**. On the other hand, stimulation of the presynaptic receptors located on the nerve terminal will regulate the monoamine release triggered by action potential, i.e. vesicular release, thereby providing a feedback mechanism that controls the concentration of the