INTRODUCTION

Epilepsy comprises a group of chronic neurological disorders characterized by repeated seizures ***(Chang and Lowenstein, 2003).*** Epileptic seizures result from abnormal, excessive or hyper synchronous neuronal activity in the brain ***(Fisher et al., 2005).*** Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter. Biochemically derived from tryptophan, 90% of serotonin is primarily found in the gastrointestinal (GI) tract. The remainder is synthesized in serotonergic neurons of the (CNS) and platelets ***(Berger et al., 2009).***

The functions of (5-HT) on the (CNS) are numerous and appear to involve control of appetite, sleep, memory and learning, temperature regulation, mood, behavior, cardiovascular function, muscle contraction, endocrine regulation, maturation of neuronal and glial cells and synaptic connections ***(Barnes and Sharp, 1999).*** There has been increasing evidence that serotonergic neurotransmission modulates a wide variety of experimentally induced seizures ***(Bagdy et al., 2007).***

The neurons of the raphe nuclei arelocated along the midline of the brainstem, & are the principal source of (5-HT) release in the brain, Axons from the neurons of the raphe nuclei form a neurotransmitter system, reaching almost every part of the central nervous system. Axons of neurons in the lower raphe nuclei terminate in the cerebellum and spinal cord, while the axons of the higher nuclei spread out in the entire brain ***(Frazer and Hensler, 1999).***

Seretonin receptors, are fourteen mammalian (5-HT) receptor subtypes which are currently recognized, and these have been classified into seven receptor families on the basis of their structural, functional and, to some extent, pharmacological characteristics ***(Hoyer et al., 1994).*** They are located on the cell membrane of nerve cells and other cell types in animals, and mediate the effects of serotonin as the endogenous ligand. With the exception of the (***5-HTR3R***) receptor, a ligand-gated ion channel, all other (5-HT) receptors are G protein-coupled, seven transmembrane that activate an intracellular second messenger cascade ***(Hannon and Hoyer, 2008).***

Generally, agents that elevate extracellular serotonin (5-HT) levels, such as 5-hydroxytryptophan and serotonin reuptake blockers, inhibit both focal and generalized seizures, although exceptions have been described. Conversely, depletion of brain (5-HT) lowers the threshold to audiogenically, chemically and electrically evoked convulsions.

Furthermore, it has been shown that several anti-epileptic drugs increase endogenous extracellular (5- HT) concentration. Seretonin receptors are expressed in almost all networks involved in epilepsies. The role of at least (***5-HT1A, 5-HT2C, 5-HT3, 5-HT7***) receptor subtypes in epileptogenesis and / or propagation has been described.

It was found that mutant mice lacking (***5-HT1A)*** or (***5-HT2C***) receptors show increased seizure activity and/or lower

threshold ***(Tecott et al., 1995; Brennan et al., 1997; Applegate and Tecott, 1998).***

In general, hyperpolarization of glutamatergic neurons by (***5-HT1A***) receptors and depolarization of GABA-ergic neurons by (***5-HT2C***) receptors as well asantagonists of (***5-HT3***) and (***5-HT7***) receptors decrease the excitability in most, but not all, networks involved in epilepsies. Imaging data and analysis of resected tissue of epileptic patients, and studies in animal models all provide evidence that endogenous (5-HT), the activity of its receptors, and pharmaceuticals with serotonin agonist and/or antagonist properties play a significant role in the pathogenesis of epilepsies ***(Bagdy et al., 2007).***

Moreover, serotonin neuro-transmission is increasingly recognized as involved in temporal lobe epileptogenesis. Evidence arising from studies performed in experimental animal models ***(Merrill et al., 2007),*** from human functional neuro-imaging studies ***(Theodore et al., 2007)*** and from genetic studies ***(Manna et al., 2007)*** support the relationship between 5-HT brain concentrations and epilepsy, whereas lower levels of serotonin seems to be pro-convulsant.

As small clinical trials have shown that the selective serotonin reuptake inhibitor as fluoxetine might be useful in pharmacoresistant epilepsy therapy ***(Albano et al., 2006).*** We are going to study the effect of Fluoxetine on patients with intractable focal epilepsy.

AIM OF THE WORK

To assess the antiepileptic effect of Fluoxetine on patients with intractable partial epilepsy.

Chapter 1

SERETONIN METABOLISM

Gross Anatomy

The neurons of the raphe nuclei are the principal source of **5**-HT release in the brain.There are **7** or **8** raphe nuclei, all of which are located along the midline of the brainstem, and centered around the reticular formation.Axons from the neurons of the raphe nuclei form a neurotransmitter system, reaching almost every part of the central nervous system. Axons of neurons in the lower raphe nuclei terminate in the cerebellum and spinal cord, while the axons of the higher nuclei spread out in the entire brain ***(Frazer and Hensler, 1999).***

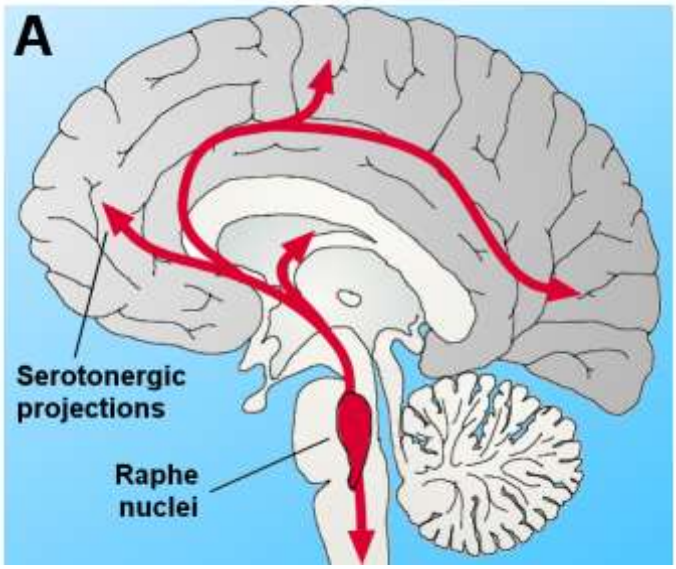

Figure (1): Diagram of the human brain showing the divergent serotonergic projections of the raphe nuclei to both cortical and subcortical locations throughout the brain ***(BILZ0R, 2005).***

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter. Biochemically derived from tryptophan, serotonin is primarily found in the gastrointestinal (GI) tract, platelets, and in the central nervous system (CNS) of animals and humans ***(Young, 2007).***

Functions of Seretonin on the CNS are numerous. They are involved in controlling sleep, apetite memory and learning, temperature regulation, mood, behaviour, cardiovascular function, muscle contraction, endocrine regulation, maturation of neuronal and glial cells and synaptic connections. It has a similar chemical structure with tryptamine, dimethyltryptamine, melatonin and bufothein belonging to the group of indolalkylamins ***(Doggrell, 2003).***

Serotonin acts directly on receptors and can also signal through a non-receptor mechanism called serotonylation, in which serotonin modifies proteins ***(Paulmann et al., 2009).***

The enzyme transglutaminase (TG) catalyzes the transamidation of serotonin to a protein (glutamyl-amide) bond residue, resulting in activation of the G protein ***(Guilluy et al., 2007).*** This process affects thrombocytes in modification of signaling enzymes called GTPases that then trigger the release of vesicle contents by exocytosis ***(Walther et al., 2003).***

Synthesis and metabolism:

Serotonin is synthesized in a two-step reaction, from tryptophan to **5**-hydroxytryptophan (**5**-HTP) and then to **5**-hydroxytryptamine (serotonin, **5**-HT).

The latter step is catalyzed by aromatic amino acid decarboxylase, while the first step is catalyzed by tryptophan hydroxylase, the rate-limiting enzyme in serotonin synthesis. Tryptophan Hydroxylase (TPH) is present in the brain mostly in its second isoform, TPH**2** ***(Gutknecht et al., 2009).*** The enzyme uses Fe**2+** as a cofactor, and oxygen and tetrahydrobiopterin (BH**4**) as co-substrates. In vitro evidence suggests that the autoregulation of the firing rate of serotonergic neurons depends on sustained tryptophan hydroxylase activity ***(Evan et al., 2008).*** L-tryptophan is transported through the blood-brain barrier into the brain using the neutral amino acids transmitter, on which competes with other amino acids – (phenylalanine, leucine and methionine).

Tryptophan- hydroxylase is the first step and speed limiting factor of **5**- HT synthesis. This enzyme was found in the brain only in the serotoninergic neurons. It enables conversion of tryptophan into **5** hydroxytryptophan, followed by the decarbolization mediated by aromatic L-amino acid decarboxylase onto **5**-hydroxytryptamine ***(Berger, 2009).***

The extracellular levels of serotonin must be tightly controlled by uptake into astrocytes and presynaptic neurons in

order to fulfill the behavioral and physiological functions of serotonin. Several mechanisms are responsible for this uptake. The most well known and well studied of these mechanisms is transport by a high affinity, sodium- and chloride-dependent transporter, Seretonin transporter (SERT) ***(Tripp and Sibille, 2010).*** This protein is highly concentrated in the raphe nuclei and in the prefrontal cortex, as well as in thalamocortical afferents ***(Qian et al., 1995).***

Seretonin transporter (SERT) is the target of serotonin selective reuptake inhibitors (SSRIs), and acute administration increases serotonin release preferentially in the raphe nuclei ***(Malagié et al., 1995).***

In addition to the high-affinity sodium-dependent SERT, a low-affinity sodium independent transporter has been described, a system called uptake**2**. Uptake**2** is a high-capacity, low-affinity transport system for monoamines that is acutely inhibited by corticosteroids ***(Hill et al., 2010).***

Two proteins mediate uptake**2** in the CNS, the plasma membrane monoamine transporter (PMAT) and the organic cation Transport transporter **3** (OCT**3**). Human OCT**3** is the major uptake**2** transporter of histamine, epinephrine, and norepinephrine, while human PMAT is the major uptake**2** transporter for serotonin and dopamine ***(Duan and Wang, 2010).***

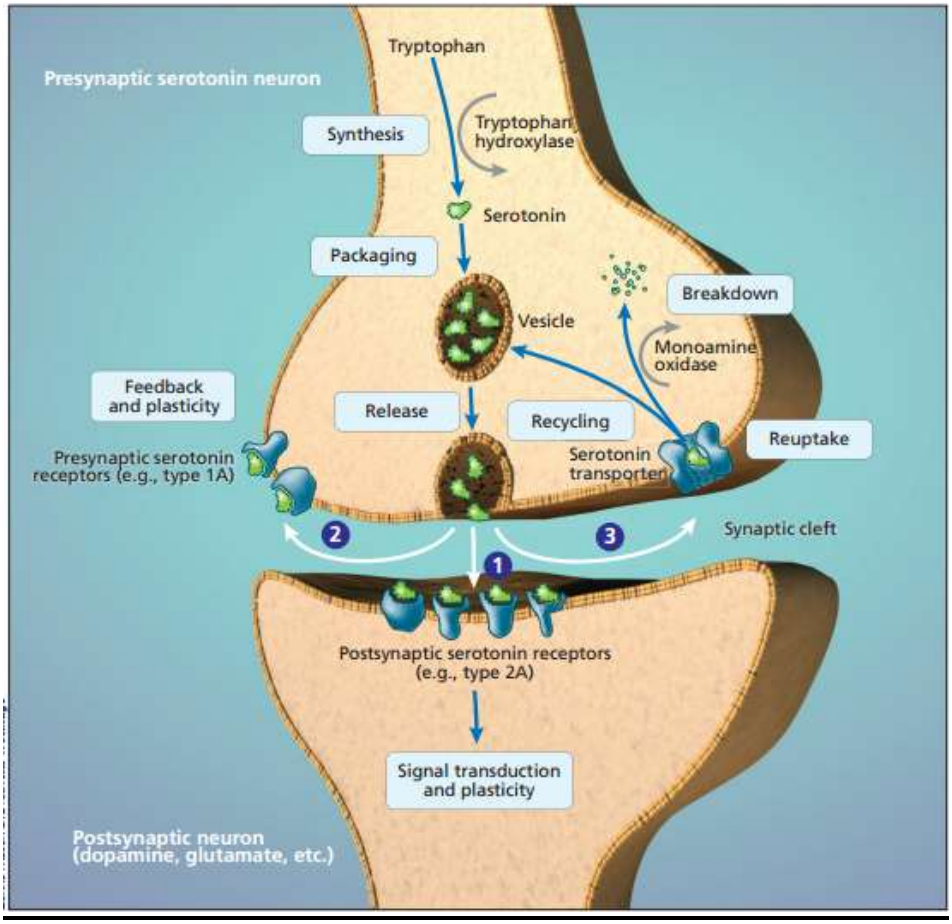

Figure (2): The serotonin synapse.

Serotonin is synthesized from tryptophan by the enzyme tryptophan hydroxylase. Serotonin is then packaged into vesicles for release into the synaptic cleft, which occurs when there is sufficient stimulation of the neuron. Serotonin released from the serotonin neuron into the synaptic cleft has multiple actions. (**1**) Serotonin binds to its receptors on other neurons. Activation of postsynaptic receptors results in transduction of the signal that initially stimulated the serotonin neuron. (**2**) Serotonin also binds to presynaptic serotonin receptors on the neuron from which it was released, which provides feedback and regulates plasticity of the neuron. (**3**) Serotonin is taken up back into the presynaptic serotonin neuron by the serotonin transporter. Serotonin is then recycled for future release or broken down by monoamine oxidase and excreted in urine (figure **2**) ***(Aan het rot et al., 2009).***

Chapter 2

SERETONIN RECEPTORS IN RELATION TO EPILEPSY

In the recent **20** years, seven distinct families of **5**-HT receptors have been identified (Table **1**) and various subpopulations have been described for several of these ***(Nichol and Nichols, 2008).***

All **5**-HT receptors, except the ***5-HT3*** subtype, which is a ligand gated ion channel, belong to the large family of seven transmembrane spanning, guanine nucleotide triphosphate (GTP)-binding protein (Gprotein) coupled receptors. When ligand binds to these receptors, the associated heterotrimeric G-protein complex dissociates into alpha and beta, gamma subunits. These subunits then activate down stream effector pathways until GTP hydrolysis occurs and the subunits reassociate with the receptor in ***(Wess, 1997)***. However the most relevant receptors in epilepsies are: ***5- HT1A, 5-HT2C, 5-HT3*** and ***5-HT7*** receptors respectively.

Table (1): The classification and their signal pathways of **5**-HT receptor Subtypes ***(Barnes and Sharp, 1999).***

Receptor		Major signal pathway	Other G-proteins	Main signal pathways	Agonists**	Antagonists**
5-HT$_1$	5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{1D}$, 5-HT$_{1F}$	Gi/o	Gz	↓ cAMP	8-OH-DPAT, Buspirone Anpirtoline, CP 94253	WAY-100635 SB-224289, GR-127935
5-HT$_2$	5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$	Gq/11	Gi/o, G12, and G13	PLC, Ca^{2+}, and PKC (+)*	DOI, m-CPP, RO 600175	Ketanserin, M100907 Mesulergine, SB-200907
5-HT$_3$	5-HT$_{3A}$, 5-HT$_{3B}$, 5-HT$_{3C}$, 5-HT$_{3D}$, 5-HT$_{3E}$	Ion channel	–	Depolarization	mCPBG, 2-CH3-5-HT	Ondansetron, Tropisetron
5-HT$_4$	5-HT$_{4A}$ - 5-HT$_{4D}$, 5-HT$_{4E}$	Gs	G13	↑ cAMP	BIMU-8, RS 67333, Cisapride	GR-113808, SB-204070
5-HT$_5$	5-HT$_{5A}$, 5-HT$_{5B}$	Gi/o	NT	↓ cAMP	–	–
5-HT$_6$	–	Gs	NT	↑ cAMP	EMDT, WAY 181187, WAY 208466	SB-399885
5-HT$_7$	5-HT$_{7A}$, 5-HT$_{7B}$, 5-HT$_{7C}$, 5-HT$_{7D}$	Gs	G12	↑ cAMP	8-OH-DPAT	Amisulpride, SB-269970

5-HT**1** receptors:

This group consists of five receptor subtypes (***5- HT1A, 5-HT1B, 5-HT1D, 5-HT1E*** and ***5-HT1F***). There is no ***5-HT1C*** receptor, as it was reclassified as the ***5-HT2C*** receptor. They are mostly (but not exclusively) associated with Gi/G**0** proteins and inhibit production of cAMP. Fully functional ***5-HT1A, 5-HT1B*** and ***5-HT1D*** receptors have been found in many tissues of various species ***(Hoyer and Martin, 1998).***

The ***5-HT1A*** receptor is the most extensively distributed of all the **5**-HT receptors. In the central nervous system, ***5-HT1A*** receptors are present in high density in the cerebral cortex, hippocampus, septum amygdala, and raphe nucelus, but they were proven in small amounts in the basal ganglia and thalamus as well ***(El Mestikawy et al., 1993).*** However, they

can be found also in myentericus plexus and whole gastrointestinal tract.

In the brain, ***5- HT1A*** receptors act as autoreceptors as well as postsynaptic receptors. They are involved in the inhibition of "discharge" of neurons, regulation of the production of ACTH (but not prolactin), and regulation of behavior and eating ***(Wang et al.,*** *2009)***.** They play probably an important role in the emergence of anxiety ***(Klemenhagen et al., 2006).*** Moreover, ***5-HT1A antagonists*** (buspiron, gepiron) are used or developed for the treatment of anxiety and depression ***(Artigas et al., 2006).*** The antianxiety actions of ***5-HT1A*** (partial) agonists may provide primarily presynaptic somatodendritic ***5- HT1A*** receptors (leading to reduced release of **5**-HT in terminal areas), whereas the antidepressant action of **5**- HT**1**A agents may primarily provide postsynaptic ***5-HT1A*** receptors ***(De Vry, 1995).***

The function of the is ***5-HT1E*** receptor is unknown due to the lack of selective pharmacological tools, It is hypothesized that the ***5-HT1E*** receptor is involved in the regulation of memory in humans due to the high abundance of receptors in the frontal cortex, hippocampus and olfactory bulb, all of which are regions of the brain integral to memory regulation ***(Shimron- Abarbanell et al., 1995).***

The 5-HT1F receptor exhibits intermediate transmembrane homology with several other ***5-HT1*** receptors.

Agonist effects of **5**-HT were antagonized completely and apparently competitively by the nonselective **5**-HT antagonist methiothepin ***(Adham et al., 1997).*** Detection of ***5-HT1F*** receptors in the uterus and coronary arteries suggest a possible role in vascular contraction ***(Nilsson et al., 1999).*** Although distribution in the brain appears limited, there are distributional similarities with **5**-HT**1**Dβ receptors ***(Bhalla et al., 2002).***

5-HT**2** receptors:

This class has three subtypes – ***5-HT2A***, ***5-HT2B***and ***5-HT2C***, showing **46-50** % structural homology, This is the main excitatory receptor subtype among the G-protein coupled receptors for serotonin (**5**-HT).Although ***5-HT2A*** may also have an inhibitory effect on certain areas such as the visual cortex and the orbit frontal cortex ***(Hannon and Hoyer, 2002).***

5-HT2A receptor is expressed in many central and peripheral tissues &mediate the contraction answer of smooth muscles. Furthermore, increased platelet aggregation and increased capillary olanzepine permeability following exposure to serotonin ***(Cook et al., 1994).***

In the CNS, ***5-HT2A*** receptors are present mainly in the crust, claustrum and basal ganglia. Activation of ***5- HT2A*** receptor leads to stimulation of secretion of ACTH, corticosterone, oxytocin, renin, and prolactin ***(Bortolozzi et al., 2005).*** Inhibition of ***5-HT2A*** receptor influences

behavior. ***5-HT2A*** antagonists with different receptor binding affinity (risperidone, ritanserine, seroquel, etc.) are used or are being developed for the treatment of schizophrenia ***(Kim et al., 2009).*** Recent studies suggest that ***5-HT2A*** receptors may play a more prominent role in the behavioral actions of hallucinogens than ***5-HT2C (Chang et al., 2009).***

5-HT2B imunoreactivity was detected in the cerebellum, lateral septum, hypothalamus and medial part of the amygdala ***(Cox et al.,*** *1995****).*** Activation of ***5-HT2B*** receptor leads to contraction of smooth muscle of stomach fundus. Direct injection of a selective agonist in amygdala have anxiolytic effects in rats. While antagonists of *5-HT2B* receptors (e.g. SB **200646**) are relatively new and may find clinical application in the treatment and prevention of migraine ***(Kennet et al., 1998).***

The knowledge of***5-HT2C*** action remains limited. Mutant mice lacking the ***5-HT2C*** receptor subtype are extremely susceptible to audiogenic seizures and are prone to spontaneous death from seizures, suggesting that serotonergic neurotransmission mediated by ***5-HT2C*** receptors suppresses neuronal network hyperexcitability and seizure activity ***(Applegate and Tecott, 1998).***

Fluoxetine and other SSRIs stimulate ***5-HT2C*** function indirectly by increasing the level of serotonin in the synapse. Fluoxetine does also act as a direct ***5HT2C*** antagonist. In contrast, through inhibition of reuptake, in some atypical