Chapter I

INTRODUCTION

Neurotransmitters (NTs) are small molecules that are liberated by a presynaptic neuron into the synaptic cleft and cause a change in the postsynaptic membrane potential by acting on a specific receptor. This change can be either a direct depolarization or hyperpolarization, or the activation of second messengers that eventually lead to changes in firing rate. These transmitters carry messages from one part of the brain to another, essentially functioning as a communication system within the brain itself. Without neurotransmitters, the brain cannot receive clear and coherent signals. It is now increasingly accepted that a nerve cell can produce more than one neurotransmitter (Leonard, 1984) but each neurotransmitter has a particular biosynthetic pathway and can be divided into three principal classes, since they share some enzyme or metabolic pathway. The first class is made up of acetylcholine alone, the second class is the biogenic amines that are molecules formed by an amino acid losing a hydroxyl or carboxyl group. The third class is made up of amino acids per se (Table 1.1).

Table 1.1: The Classical Neurotransmitters (Bloom, 1996)

- Acetylcholine
- Amino acids (glutamate, Glycine, γ-Amino Butyric acid (GABA))
- Monoamines
  - Catecholamines—dopamine (DA), noradrenaline/norepinephrine (NE)
  - Indolamine—serotonin (5-HT), melatonin.
Neurotransmitters can act as inhibitory or excitatory signals to the postsynaptic cell, by hyperpolarizing or depolarizing its membrane, although the same molecule can function as an inhibitor or an excitator. This happens because there are a small number of neurotransmitters but a great variety of their receptors on different types of cells. Acetylcholine, for instance can act as an excitator when it binds to one type of receptor, and as an inhibitor when bound on another kind, even if both types of receptors are present in the same cell. The following is a list (Table 1.2) of several known and well studied neurotransmitters.

Table 1.2: Nature of Different Neurotransmitters (Bloom, 1996)

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Function</th>
<th>Synthesis by (enzymes)</th>
</tr>
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<tbody>
<tr>
<td>Acetylcholine</td>
<td>mostly excitatory</td>
<td>Choline acetyltransferase</td>
</tr>
<tr>
<td>Bioactive amines:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>Excitatory and inhibitory</td>
<td>Tyrosine hydroxylase</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Excitatory</td>
<td>Tyrosine hydroxylase and dopamine-β-hydroxylase</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Excitatory and inhibitory</td>
<td>do</td>
</tr>
<tr>
<td>Serotonin</td>
<td>mostly inhibitory, partly excitatory</td>
<td>Tryptophan hydroxylase</td>
</tr>
<tr>
<td>Amino acids:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>Excitatory</td>
<td>Metabolic amino acid</td>
</tr>
<tr>
<td>Glycine</td>
<td>mostly inhibitory</td>
<td>Metabolic amino acid</td>
</tr>
<tr>
<td>γ-Aminobutiric acid</td>
<td>Inhibitory</td>
<td>Glutamate decarboxylase</td>
</tr>
<tr>
<td>(GABA)</td>
<td></td>
<td></td>
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</tbody>
</table>

Thus neurotransmitters are small molecules that ferry information from the end of one nerve to the "beginning" of another by activating a large molecule at the far end of the synapse called a receptor. Acetylcholine was the first neurotransmitter discovered. Acetylcholine neurons convey sensory information to the brain and control muscular
tension, including peristalsis. Cholinergic neurons are also present in the central nervous system (CNS).

The catecholamines [epinephrine (adrenalin), norepinephrine and dopamine] control so-called adrenergic systems in the CNS. Some of these neurons radiate from the limbic system (emotional centers) and discharge neurotransmitters in a diffuse manner into the frontal cortex, i.e. into broad areas of brain tissue as opposed to delivering the chemical to specific synapses. They thus account for "global vigilance" (staying awake), mood, fight or flight response, etc. In addition they act peripherally to modulate blood pressure and other functions. These compounds are in turn controlled by peptide compounds secreted from the hypothalamus and thyroid.

Serotonin is the primary inhibitory neurotransmitter modulating the excitatory catecholamine systems in the CNS. Serotonin neurons control memory, mood, sex drive, etc. The compound has many other functions including allergic response, and regulation of vasotension, especially in the meninges and other brain tissues. It is most highly concentrated in the gut. Melatonin, synthesized biogenically from serotonin, sets circadian rhythms, i.e. sleep cycles.

Histamine mediates allergic response and is concentrated in mast cells, whose main function is to detect trauma and release histamine and co-transmitters (leukotrienes, ATP). Another primary function of histamine is to regulate secretion of gastric acid.

Altered neurotransmitter levels are many a times cause and/or effect of many disorders, especially central nervous system disorders. These neurotransmitters often singly and at times in conjugation modulate the course of the disease. Neurotransmitters such as dopamine, serotonin and acetylcholine regulate diseases and disorders such as depression, anxiety disorders, parkinson's disease, alzheimer's disease, schizophrenia.
and epilepsy. While serotonin plays a very important role in depression and anxiety, dopamine depletion enhances parkinson's disease. Epilepsy may develop because of an abnormality in brain wiring, an imbalance of nerve signaling chemicals called neurotransmitters, or some combination of these factors.

Epilepsy has been recognized for over 2000 years. Hippocrates recognized epilepsy as a brain disorder. However, it took more than two millennia for this fact to gain wide acceptance. Today epilepsy is viewed as a symptom of disturbed electrical activity in the brain caused by a variety of disorders. It is a collection of many different types of seizures that vary widely in severity, appearance, cause, consequence, and management (Meldrum, 1990).

Broadly speaking, epilepsy is the chronic condition in which seizures recur periodically (Sander and Shorvon, 1987; Garnett, 1989). Seizures occur as a result of a disruption in the normal firing and resting patterns of the neuron. There may be a collection of damaged neurons, or a chemical disruption may occur.

The idea in general prevails that having an understanding of the biochemical basis of epilepsy is not essential to being involved in the patient's care. But overwhelming literature have shown that of the several factors that influence the pathogenesis of epilepsy complex neurotransmitter mechanism is probably one of the factors involved during paroxysmal disorders.

A dramatic progress in the understanding of the neurobiology of central nervous system disorders have focused the role of number of brain areas, neurotransmitters and neuropeptides in the CNS that regulate body homeostasis by their own hyper and hypo activity. In this context, sufficient role of amygdala and hippocampus (Shouse et al,
2001 a; Chen et al, 2001; Fernandez-Guardiola and Fernandez-Mas, 1995; Broderick et al, 2000), thalamic and hypothalamic area (Horn and Gehring, 1996), neocortical region (Holmes, 1994) and the central dopaminergic system (Bo et al, 1993; Starr, 1996; Blows, 2000), locus coerules with central noradrenergic pathways (Clough et al, 1994; Meierkord et al, 1994; Ferraro et al, 1994 a), serotonergic (Yan et al, 1994; Blows, 2000), histaminergic (Feng and Faingold, 2000) and the inhibitory GABAergic system (Kokaia et al, 1994 a; Bartholini, 1985) have been documented.

All these findings have long put the concept of falling sickness into recycle bins and have accepted epilepsy as a clinical disorder. Knowledge about the disorder has grown enormously in the recent years and that has been possible because of the extensive researches going around the world both in human subjects and animal models.

The Spectrum of Experimental Epileptic Models

Animal models of neurological deficits are essential for the assessment of new therapeutic options. A wide variety of animal models of epilepsy and epileptic seizures exist. Experimental epilepsy models are developed to assess the physiopathology of epileptic seizures (Biziere and Chambon, 1987). A coordinated experimental design is pivotal for the understanding of epileptic networks and allows developing new concepts for rational drug design. Although much progress has been made in the analysis of human surgical specimens, such studies are usually restricted to a late stage of the disease and must take into account different clinical histories of patients as well as lack of appropriate control material. In this respect, animal models provide important adjunct tools to address basic mechanisms of focal epileptogenesis as well as antiepileptic drug (AED) treatment/resistance (Löscher, 2002). The tremendous diversity of available animal models offers both opportunity and challenge.
Animal models with epilepsy can be classified as: 1) experimental seizures induced by chemical convulsants or by electrical stimulation, 2) reflex epilepsies, 3) idiopathic epilepsies (Bziere and Chambon, 1987). Accordingly several seizure models are available for research like the Papio papio (Naquet et al, 1972), genetically-epilepsy prone rat (Wang et al, 1994), Krushinski-Molodkina rat (Onodera et al, 1992) and also the experimentally-induced epileptic models like – penicillin (Witte, 1994; Ferraro et al, 1994 b; Hategan et al, 1995; Stankiewicz et al, 1995), Pentylentetrazol (Schwindt et al, 1997), pilocarpine (Starr and Starr, 1993; El-Etri et al, 1993), amygdaloid kindling (Shouse et al, 2001 a, b) etc. each of these in vivo models results in the development of spontaneous seizures, which makes them valuable for investigating human epilepsies, epileptogenesis and the prevention of epileptogenesis. It has been suggested that rats are not as appropriate as primates for the symptomatic modelling of diseases, but a large body of data argues against this view. Advances have been made in identifying rat equivalents of akinesia, tremor, postural deficits and dyskinesia and today studies in rodents are considered as effective models that complement studies in primates (Cenci, 2002). And today chemical (pilocarpine, penicillin, pentylentetrazol etc) induced/ electrical stimulated/genetic epileptic rat models are the most used investigative models in epilepsy research.

Keeping in mind all these information and also the fact that epilepsy by definition is a chronic disorder, or at least a disorder that manifests itself over a period of time, not simply at an instant, the present work has been based primarily on two functionally different chronic models (repeated intracortical penicillin induced and amygdaloid kindled). Work has been carried out on two acute models (single penicillin and pentylentetrazol induced) as well, so as not to miss the immediate manifestations of the disorder.
Of the three models, penicillin induced epileptogenesis is an experimental model of human generalized epilepsy (Assai et al, 1995).

Pentylenetetrazol induced seizure is widely used as a standard model for myoclonic and absence (petitmal) seizures (Lösch and Schmidt, 1998).

Kindling preparation is considered to be a very powerful tool for the pathophysiological study of epilepsy as it is an adequate analogue of human temporal lobe epilepsy and complex partial seizures with secondary generalization, characterized by the increase in electrographic afterdischarge duration (Sato et al, 1990; Goddard, 1969; Girgis, 1981; McNamara, 1984).

Therefore in the present investigation these three representative epileptic rat models were selected to explore how some of the neurotransmitters function during epileptogenesis.

The overall purpose of this study is to learn about the relation between epilepsy and several brain neurotransmitters.

Therefore the Objectives of the present study were:

1) Quantitative analysis of different neurotransmitter levels especially norepinephrine, dopamine, serotonin, histamine.

2) To analyse RNA and DNA fragmentation in different brain areas of normal (control) and epileptic rat models.

3) A critical analysis of behaviour patterns and EEG recordings in experimental epileptic rats following application of penicillin, pentylenetetrazol and amygdaloid kindling.

4) A detailed understanding of the histomorphology, cell structure, cell volume, nuclear volume, fibre connections of different brain areas in normal and in experimental animals following identical induction of epileptogenesis.