EFFECTS OF ANXIO-SELECTIVE DRUG (BUSPIRONE) ON BRAIN NEUROTRANSMITTERS

THESIS

Submitted in Partial Fulfilment For M.D. Degree in Pharmacology

BY

Ahmed Nour El-Din Hassan
M.B.,B.Ch. (1984); M.Sc. Pharmacology (1990)
Assistant Lecturer in Pharmacology
Ain Shams University

Supervised by

Prof. Dr. Ahmed Shaaban Abou Seif
Professor of Pharmacology

Ass. Prof. Dr. Osama Mahmoud El Sirafy
Assistant Professor of Pharmacology

Faculty of Medicine
Ain Shams University
1995

Acknowledgement

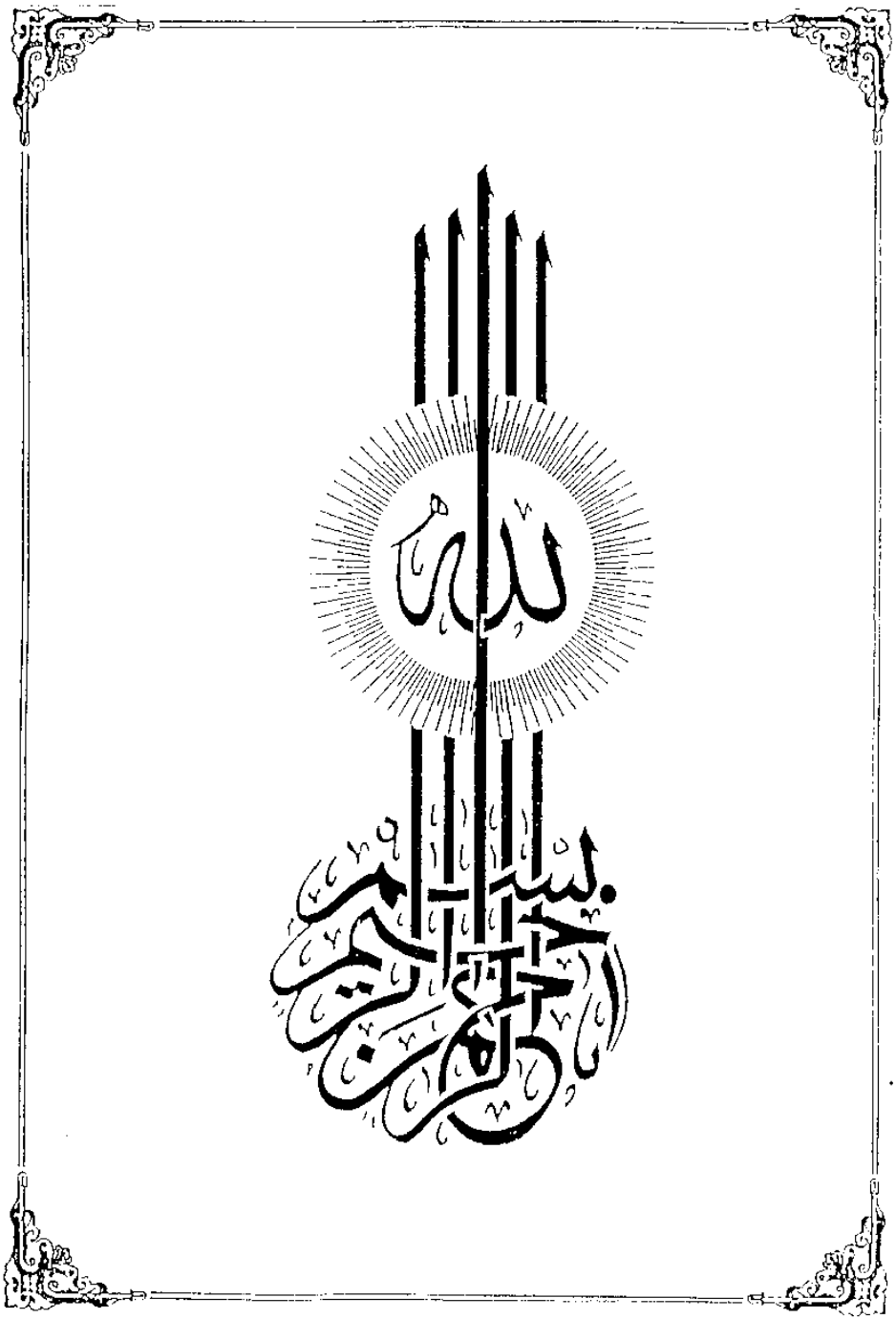
I wish to offer my sincere thanks and gratitude to Prof. Dr. Ahmed Shaaban Abou-Seif, Professor of Pharmacology, Faculty of Medicine, Ain Shams University, for his effort, continuous guidance and valuable help throughout the present work.

I would like also to thank Dr. Osama Mahmoud El-Serafy, Assistant Professor of Pharmacology, Faculty of Medicine, Ain Shams University, for his kind assistance and valuable criticism thoughout this work.

I wish to express my deepest obligation and thanks to Prof. Dr. Adel Disawy Bakry, Professor of Pharmacology, Faculty of Medicine, Ain SHams University, for previous supervision and planning of this work.

I wish also to express my thanks to Prof. Dr. Mishaq Salama Abou-Zeid, Professor of Pharmacology, Faculty of Medicine, Ain Shams University, for previous supervision of this work.

I want also to express my sincere gratitude to Dr. Hanaa Moky El-Din, Lecturer of Pharmacology, Faculty of Medicine, Ain Shams University, for his valuable advice and cooperation in the practical part of this thesis.



I am also much obliged to Prof. Dr. Mohamed Ibrahim Rushdy, Professor of Evaluation and Analysis, Petroleum Research Institute for his valuable advice as regard the chromatographic analysis.

Finally, I wish to offer my thanks to all my colleagues in Pharmacology Department who shared me this work for years and also to Bristol-Meyers Squibb, Scientific Office, Egypt, for their references supply.

CONTENTS

INTRODUCTION

Anxiety disorders are a leading psychiatric problem allover the world today, affecting approximately 3 - 5 % of the population (*Lader, 1994*). Every one experiences anxiety at sometime in life and, in fact, a mild degree of anxiety is frequently constructive since it motivates and often increases alertness. However, more severe anxiety may be counter-productive and frequently producing somatic symptoms and when anxiety reaches this proportion, it can be classified as an anxiety disorders (*Goldberg, 1984*). There are several forms of anxiety which produce a wide range of either psychological symptoms as sense of fearful anticipation and disturbed sleep, or physical symptoms as palpitation, diarrhea, impotence, headache, dizziness and paraesthesia (*Gelder, 1991*). Anxiety accompanies almost every psychiatric disorder as schizophrenia, depression and mania, and is a common component of numerous organic disorders as dysrrythmias, myocardial infarction, thyrotoxicosis and seizure (*Wincor, 1992*). In the revised third edition of the **Diagnostic and Statistical Manual of Mental Disorders** (**DSM-III-R**) (*1987*), primary anxiety disorders have been divided into a number of specific types (*Appendix* 1).

The ideal anxioselective compound would act directly on the neural substrates responsible for anxiety with reduction in anxiety without concomitant pharmacological effects, whether they are deleterious side effects or potentially

useful ancillary properties (*Eison, 1984*). For thirty years ago, the benzodiazepines were hailed as a breakthrough of antianxiety drug therapy (*Janicak et al., 1993*). At the same time they are still sedatives, control convulsions and produce muscle relaxation, properties that are unneeded in treatment of anxiety (*Taylor, 1988*). They possess a variety of well known side effects, including drowsiness, impaired cognitive and motor function, potentiation of other CNS depressants and in some patients induce depression (*Gammans et al., 1992*). In addition, abuse liability and occupance of withdrawal manifestations after chronic use are encountered (*Lader, 1985*).

The introduction of buspirone in the United States in 1986 marked the advent of a new class of anxiolytic agents, that differs both chemically and pharmacologically from the benzodiazepines (*Riblet et al., 1984*). Buspirone is the first 5-HT_{1A} partial agonist to be widely used in clinical practice with anxiolytic activity comparable to that of benzodiazepines without their ancillary side effects (*Taylor, 1988*). One of the particular interest is that this partial agonist has also therapeutic potential for treating both major depression and mixed anxiety-depression syndromes (*Robinson, 1989*). As it has been estimated that about one third of patients with a primary anxiety disorder will fulfill the diagnostic criteria for depression disorders at sometime during the course of their illness, while approximately two thirds of depressive patients will meet the criteria for an anxiety disorder (*Maser and Cloninger, 1990*), Behavioral, biochemical and electrophysiological studies suggest that buspirone interacts with monoaminergic

and GABAergic systems in a manner different from that of the benzodiazepines (*Eison and Temple, 1986*). Advances in technology such as chromato-graphic techniques now allow to detect quantitatively small, but functionally significant alternations in neurotransmitter turnover (*Eison, 1989*), and it is used in an attempt to reaxamine old problems in new ways.

There are basically three ways of looking for a role of neurotransmitters in psychiatric disorders. One approach is to search for an abnormality in CNS neurotransmitters in patients with anxiety, secondly by using animal model for anxiety and to investigate neurotransmitter characteristics in the brain of such animals, finally by using accepted anxiolytic agents and finding out their effects on brain neurotransmitters or other biochemical or biological inter-actions with the brain (*Braestrup and Nielsen, 1982*).

5-Hydroxytryptamine

In the late nineteenth century, scientists had been aware that a substance found in the serum caused powerful contraction of smooth muscle organs, and later on enteramine was identified in the gut. At 1948, the vasotonic substance in the serum was identified as "serotonin" and rapidly shown to be identical with enteramine (*Rapport et al., 1948*). The presence of serotonin in mammalian brain and the heterogenous distribution in dog brain led to the suggestion that it might act as a neurotransmitter in the CNS (*Bogdanski et al., 1956*). Only about 1 - 2 % of the serotonin in the whole body is found in the brain, and as it cannot cross the blood brain barrier, it is clear that brain cells must synthesize their own transmitter.

Evidence for the existence of serotonergic receptors was first presented at 1957, and classified as D-receptors for those blocked by dibenzyline, and M-receptors, for those blocked by morphine (*Gaddum and Picarelli, 1957*). *Peroutka and Snyder (1979)*, proposed the existence of two classes of serotonergic receptors, 5-HT_1 and 5-HT_2 labeled by high affinity towards [^{3}H] 5-HT and [^{3}H] spiperone respectively with suggestion that 5-HT_1 might be heterogenous. These results were confirmed by *Pedigo et al., (1981)* who proposed the existence of two sites labeled by [^{3}H] 5-HT_{1A} and 5-HT_{1B} with relatively high affinity of neuroleptic spiroperidol towards 5-HT_{1A}. Later on *Pazos et al., (1985)* supposed the presence of 5-HT_{1C} binding site.

Bradley et al., (1986) proposed three major serotonergic receptor classes : 5-HT_1-like, 5-HT_2 and 5-HT_3. 5-HT_2 was proposed for the D-receptors and 5-HT_3 corresponded to the M-receptors that had been described much earlier. This classification was later slightly modified by *Peroutka (1990)* after introduction of 5-HT_{1C} and 5-HT_2 receptors. He proposed three main classes of receptors : 5-HT_1 (including 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} subtypes), and 5-HT_3. Although useful, this classi-fication, which was based on agonist and antagonist drug specificity, did not account for many receptors such as, 5-$HT_{1nonA, nonB, non C}$; 5-HT_{1P}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, the last two have similar pharmacological properties but encoded by two different genes. Also there are several potential receptors such as 5-HT_{1E}, 5-HT_{1S} and 5-HT_{1R} (*Zifa and Fillion, 1992*). On the other hand, potential receptors that mediate 5-HT activities have a pharmacological profile different from that of the following known 5-HT receptors as 5-HT_4 stimulating the adenyl cyclase activity (*Dumuis et al., 1988*).

Hartig (1989) proposed to classify the 5-HT receptors on the basis of their structural homology, which had been established by molecular biology, and their predominant transduction system. He distinguished serotonergic receptors, the activity of which is coupled to a G-protein, from those directly linked to an ionic channel. Of the former, the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and 5-HT_4 receptors were associated with adenyl cyclase, whereas the 5-HT_{1C} and 5-HT_2 receptors were associated with phospholipase C (PLC). Also, there are several potential receptors still awaiting appropriate classi-fication, e.g. 5-HT_{1E} sites, 5-HT_{1S} sites, 5-HT_{1R}

sites, receptors inhibiting the release of endogenous aspartate, 5-HT receptors depolarizing neonatal rat motoneurons and receptors coupled to cyclic GMP production (*Zifa and Fillion, 1992*). Another receptors include : receptors enhancing a hyper-polarization-activated cationic current (*Pape and Mc cormick, 1989*), and receptors mediating slow excitatory responses in neurons of CA1 region of the hippocampus (*Chaput et al., 1990*). The pharmacological and functional properties of some serotonergic receptors will be mentioned briefly.

5-HT_{1A} Receptor Subtype :

The distribution of 5-HT_{1A} receptors within the brain has been studied in many animal species, and the highest density was observed in the limbic system : hippocampus (dentate gyrus and CA1), septum, amygdala and the cortical limbic area (*Zifa and Fillion, 1992*). The preferential distribution of 5-HT_{1A} receptors in limbic areas is consistent with their involvement in the control of mood and anxiety. 5-HT_{1A} receptors are highly localized in raphe dorsal and median nuclei where act as somatodendritic autoreceptors regulating the firing of serotonergic neurons (*Hillegaart, 1991*). While 5-HT_{1A} receptors located in raphe nuclei are presynaptic (*Verge et al., 1985*), they are localized postsynaptically in hippocampal pyramidal cells (*Verge et al., 1986*) with suggestion that some of the receptors are not located on serotonergic neurons or glial cells.

Although the negative coupling of 5-HT_{1A} receptors with adenyl cyclase activity is well established and involves G_i-protein (*Harrington et al., 1988*), some investigators denoted that there may be also a positive coupling (*Shenker et al., 1985; Mork and Geisler, 1990*). According to the results obtained by other investigators on other receptors coupled to adenyl cyclase e.g. D_1 or B_2 adrenoceptors (*Okamoto et al., 1991; Rubenstein et al., 1991 and Federman et al., 1992*), it was concluded that, inhibition or stimulation of adenyl cyclase activity by 5-HT_{1A} may be either due to the existence of different 5-HT_{1A} receptors specifically linked to G_i or G_s or to the same receptor linked alternatively to G_i or G_s depending on phosphorylation of serine residue which enhances coupling with G_i protein, or even an inhibitory G- protein can mediate the stimulation of certain types of adenyl cyclase (type II) through ßy subunits (*Zifa and Fillion, 1992*).

The 5-HT_{1A} receptors are also directly linked to the PLC activity which is dependent on the cell type (*Liu and Albert, 1991*). Moreover, the IP_3 production was shown to be induced by 5-HT_{1A} receptors in the hippocampus (*Janowsky et al., 1984*). In addition to the coupling of 5-HT_{1A} receptors with membrane-bound enzymes (adenyl cyclase and phospholipase), these receptors also control G-protein-coupled K^+ channels, notably in the hippocampus (*Andrade et al., 1986*), in the dorsal nucleus (*Innis et al., 1988*) and the ventromedial hypothalamus (*Newberry, 1992*). It was suggested that G-protein may directly couple 5-HT_{1A} and $GABA_B$ to the same K^+ channel (*Andrade et al., 1986*).

In addition to these data, *Albert et al. (1990)* found four different sizes of mRNA in rat brain to 5-HT_{1A} receptors, with suggestion of heterogenicity of 5-HT_{1A} receptor and all 5-HT_{1A} receptors are not identical throughout the brain.

The major reason for the interest in 5-HT_{1A} receptors is their implication in psychiatric disorders, such as anxiety and depression, beside other roles such as sexual behavior, temperature regulation and feeding behavior (*Zifa and Fillion, 1992*)

5-HT_{1B} Receptor Subtype :

5-HT_{1B} receptor subtypes occur in rat and mouse brain (*Hoyer et al., 1985*). However, these receptors are not observed in guinea pig, cat, pig, calf, pigeon, frog, or humans (*Waeber et al., 1989*$_a$). *Middlemiss (1984)* showed that the release of 5-HT from serotonergic terminals was controlled by autoreceptors characterized as 5-HT_{1B}, strongly suggesting that these were homologous presynaptic receptors. However, their postsynaptic location as heterologous presynaptic receptors is also documented by the existence of a serotonergic inhibition of the acetylcholine release in synaptosomes (*Harel-Dupas et al., 1991*). The 5-HT_{1B} receptors are negatively coupled to adenyl cyclase (*Bouhelal et al., 1988*). However, the autoreceptor present on the serotonergic terminals in the hippocampus is not coupled to G_i, G_s or G_o proteins as shown by the lack of interaction with pertussis toxin and cholera toxin (*Blier, 1991*) with suggestion that 5-HT_{1B} receptor-coupling mechanisms are heterogenous.

The major function of the 5-HT_{1B} receptors is the control of 5-HT release from the serotonergic neuron terminals i.e. presynaptic homologous autoreceptors (*Middlemiss, 1984*). Also, it appears that the 5-HT_{1B} receptors may play an important role in the control of CNS function by modulating the release of other neurotransmitters at the level of the nerve terminal. 5-HT_{1B} receptors inhibit the release of acetyl-choline in hippocampus (*Harel-Dupas et al., 1991*), noradrenaline in peripheral tissues (*Molderings et al., 1990*), and possibly dopamine as shown in-vivo by microdialysis (*Benloucif and Galloway, 1991*). They also stimulate the release of prolactin in rat (*Van de Kar et al., 1989*).

5-HT_{1D} Receptor Subtype :

The existence of the 5-HT_{1D} receptor subtype was proposed by *Heuring and Peroutka (1987)* on the basis of binding studies performed with bovine brain using [^{3}H] 5-HT labeling in the presence of drugs masking 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} subtypes; the remaining binding was called SAT_{1D}. The heterogenicity of 5-HT_{1D} was studied by *Leonhardt et al. (1989)*, and could differentiate another subtype according to its affinity for certain drugs, and so called 5-HT_{1E} which was reported initially to be parallel of that 5-HT_{1D}, however, *Lowther et al. (1991)* showed, in postmortem human brain samples, that the 5-HT_{1D}/5-HT_{1E} ratio differed depending on the brain region. The existence of another receptor, 5-HT_{1R}