PHARMACOLOGICAL STUDY OF PHENYL-ALPHA-CYCLOPENTYL ACETYL GLYCINE

Thesis

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Pharmacology

by

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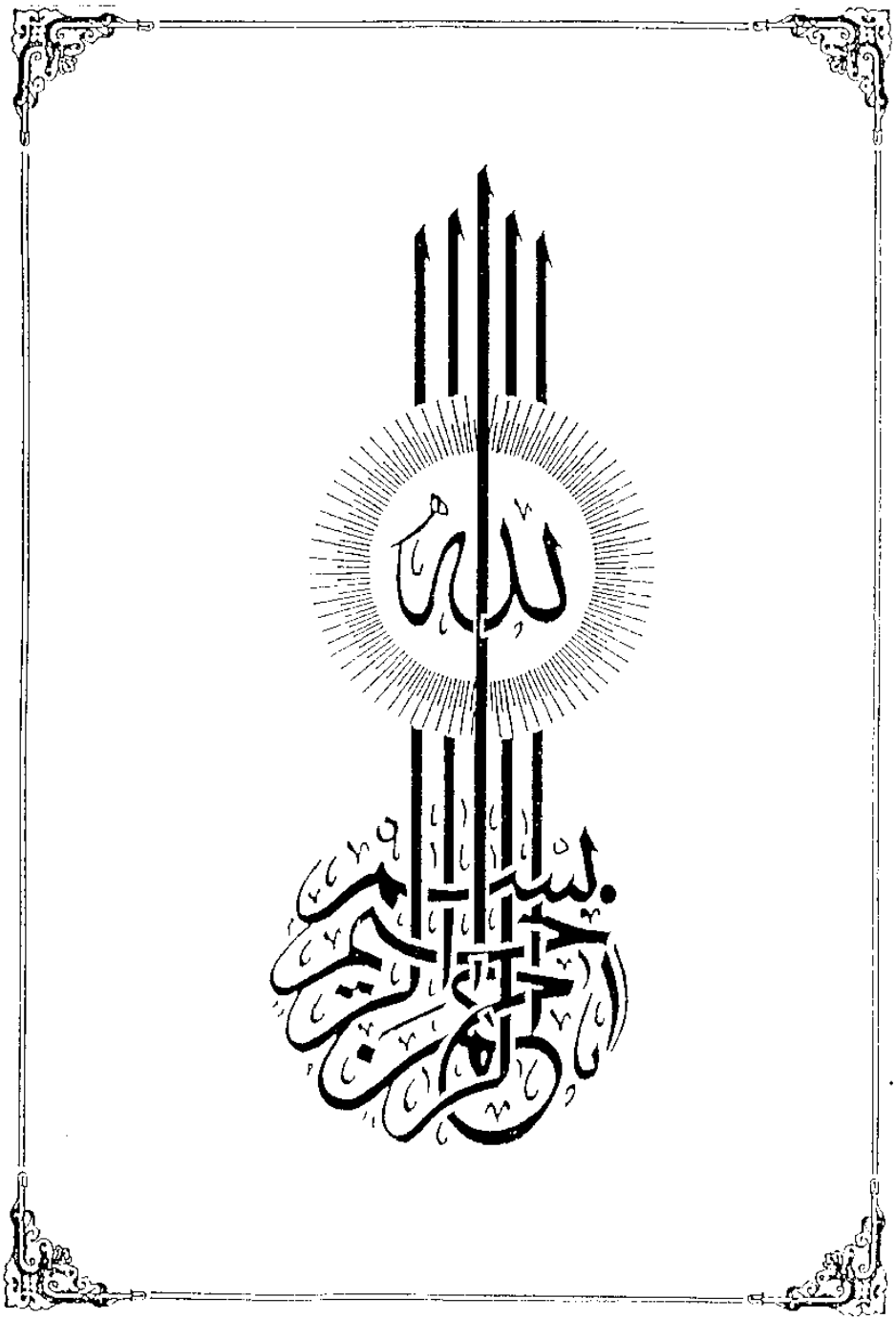

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C O N T E N T S

INTRODUCTION

I N T R O D U C T I O N

The concepts concerned with the functioning of the "Human" body have progressed from the primitive and simple inquiry about an "External", "Omnipotent" power manipulating these functions "Directly", to the recent sophisticated inquiries about microstructurs and molecules governing, not only the biological activities, but, surprisingly, also the delicate humane feelings and emotions.

The increasing interest in the role of electrolytes, peptides, amino acids etc... in explaining various physiological events, has stimulated many research workers to investigate whether these endogenous molecules would restore pathological states back to normal. The recognition of the role of endorphins in the phenomenon of pain perception and the rational use of morphine and its substitutes, has had a reputation since the previous decade (Synder, 1977). The importance of dopamine deficiency in the evaluation of the parkinsonian syndrome and the rational use of its metabolic precursor L. Dopa, in its treatment, is now well established (Kutt and McDowell, 1979). In the various fields of medicine, this approach has been the most acceptable trend in therapeutics. Insulin is the outstanding example of this trend (Williams et al., 1980).

The role of amino acids in neurotransmission in now gaining much acceptance (Iversen, 1970; Roberts and Hammerschlag, 1976). Gamma amino butyric acid (GABA) was the first amino acid shown conclusively to function as a neurotransmitter in both vertebrate and invertebrate nervous system. (Roberts and Eidelberg,1960). This assumption has been verified by numerous biochemical and immunocytochemical studies on its neural distribution and the enzyme responsible for its biosynthesis (Roberts,1976). Glutamic acid and aspartic acid have questioned as being neurotransmitters (Cooper et al., 1974).

Glycine, the simplest amino acid known($H_2N.CH_2.COOH$) is now accepted as a neurotransmitter (Johnston, 1978).

Osborne (1981) ,revised the assumptions of Brachas et al.,(1978) and postulated the following criteria as determinants of a substance to be a neurotransmitter;

1- The substance must be present in presynaptic elements of neuronal tissue, possibly in an uneven distribution throughout the brain.

2- Precursors and synthetic enzymes must be present in the neuron, usually in close proximity to the site of presumed action.

3- Stimulation of the afferrents should cause release of the substance in physiologically significant amounts.

4- Direct application of the substance to the synapse should produce responses identical to those of stimulating afferents.

5- There should be specific receptors present, which interact with the substance ; and these should be in close proximity to presynaptic structures.

6- Interaction of the substance with its receptor should induce in postsynaptic membrane permeability changes leading to excitatory or inhibitory postsynaptic potentials.

7- Specific inactivating mechanisms should exist, to stop interactions of the substance with its receptor in a physiologically reasonable frame of time.

Experiments in intact animals suggest that blood-borne glycine or its precursor serine, are not important sources for maintaining the glycine content in the C.N.S. (Aprison et al., 1970). However, within the C.N.S., either serine formed from glucose or glyoxylate formed from isocitrate can serve as a direct precursor of glycine. Serine is converted to glycine by the enzyme L-serine hydroxymethyltransferase (SHMT), which requires tetrahydrofolate as cofactor. Serine is formed from glucose by alternate pathways with 3-phosphoglycera

serving as the branch point (Fig 1). In the route via 3-phosphoserine, the rate-limiting enzyme may be 3-phosphoglycerate dehydrogenase; in alternate route via glycerate, the key enzyme may be D-glycerate dehydrogenase (Roberts and Hammerschlag, 1976). When glycine levels, as well as activities of both of these enzymes, were measured in various regions of brain and spinal cord, the D-glycerate dehydrogenase activities showed a correlation coefficient with glycine levels of approximately 0.8, whereas the corresponding value for 3-phosphoglycerate dehydrogenase was only 0.07 (Uhr and Sneddon, 1972). The latter results, together with observation that glycine is a relatively potent noncompetitive inhibitor of D-glycerate dehydrogenase, suggest that the activity of this enzyme may be rate limiting in the formation of a glycine precursor pool of serine and that glycine exerts feedback inhibition of its own synthesis at this step. These alternate pathways have common final step in the conversion of serine to glycine via SHMT. The regional distribution of glycine levels correlates well with activity levels of SHMT in spinal cord, with both enzyme and product present at highest levels in ventral gray matter (Daly and Aprison, 1974).

Routes of glycine catabolism include conversion back to serine via SHMT and back to glyoxylate via transamination, or a less-likely direct oxidation via D-amino acid oxidase (Roberts and Hammerschlag, 1976). However, the question of biosynthetic routes for glycine must be approached at a finer level than regional measurments of total activity of specific enzymes or total content of glycine (Shank et al., 1973). The evidence suggests strongly that glycine is an inhibitory transmitter of interneurones in the spinal cord. There is also some evidence that glycine is the inhibitory transmitter at the endings of descending fibers that inhibit the firing of Renshaw cells, and in neurones supplying the cuneate nucleus in the medulla oblongata, (Snyder and Enna, 1975). These facts, have been realised by many experiments to demonstrate the release of glycine from central neurones during nerve stimulation (Aprison, 1976; Roberts and Hammerschlag ,1976).

The effects of glycine are best observed in iontophoretic studies in spinal cord and brain stem. In higher regions of the C.N.S., such as cerebellum and cerebral cortex, neurones are insensitive to glycine. When tested on spinal motor neurones, the hyperpolerization induced by

glycine is identical with that of naturally released inhibitory transmitter. More precisely, both effects appear to be mediated by an increase in chloride permeability, and the equilibrium potentials for both processes are the same before and after the internal ionic concentration of motor neurones is altered by intracellular injection of anions that can move through the membrane (Krnjevic, 1974).

An antagonism to glycine action is seen with strychnine, a convulsant substance. Strychnine, therefore, is useful for identifying presumptive sites of glycine action, although it has multiple pharmacological actions, including blocking depressant effects of certain amines and competitively inhibiting acetycholinestrase (Roberts and Hammerschlag, 1976).

Studies of the binding of ^{3}H-strychnine to a membrane fraction from rat spinal cord membranes have suggested that glycine and strychnine bind to distinct sites which interact in a cooperative fashion (Young and synder, 1974a). The ability of a series of anions to inhibit ^{3}H-strychnine binding, correlated closely with their capacity to invert inhibitory postsynaptic potentials when injected iontophoretically into spinal motor neurones (Young and synder, 1974 b). Thus, strycnnine binding may be associated closely

with the ionic conductance mechenism for Cl^- in the glycine receptor. Although the glycine receptor has not yet been solubilized, and more definitive work remains to be done the above experiments represent the initial attempts to study the receptor-ionophore complex.

An important notion on the antagonistic action of strychnine to glycine-mediated transmission, is that the former may not only block post synaptic glycine receptors, but,in addition may possess a more potent presynaptic action through which the release of inhibitory transmitter is impaired. Such a dual action would account for the observation that inhibitory nerve stimulation in the goldfish nervous system is blocked by doses of strychnine smaller than those necessary to block the effects of glycine (Davidson,1975)

The post synaptic action of glycine is believed to be terminated by its active transport into pre-and post-synaptic neuronal sites and glia. A high affinity, sodium-dependent uptake mechanism for glycine has been demonstrated in the spinal cord but not in the higher region of the C.N.S., which is consistent with the proposed distribution of glycine releasing inhibitory neurones. No substance has been described that is a potent blocker of glycine

uptake, but ϐ- hydroxymercuric benzoate is partially effective both in inhibiting uptake and in potentiating the postsynaptic action of glycine (Curtis et al, 1970)

Synder et al.,(1977) suggested that benzodiazepines exert their muscle relaxant and anxiolytic effects by glycine-mimetic mechanisms. However, these suggestions do not explain the precise mechanism by which these glycine-memetic mechanisms occur, whether by directly stimulating glycine receptors, enhancing glycine release or preventing its uptake. The matters have become more complicated after the discovery of the benzodiazepine receptors (Cadre, 1981). The details of the relation between these receptors and glycine transmission are not determined yet. The effects of benzodiazepines on gamma amino butyric acid (GABA)-ergic transmission, mediating the sedative and anticonvulsant effects (Richter, 1981), may throw a light on the possible effects on glycine-ergic transmission. Costa and Guidotti (1979) suggested that, in the membranes of certain cells, the lipid portion conteins proteins that have specific binding sites for GABA and other proteins with specific binding sites for benzodiazepines. These two binding sites are coupled to a modulator and complex of large molecules

that may resemble associated subunits in allosteric enzymes. In this model of allosteric protein modulator for GABA function, the benzodiazepines have no direct GABA- mimetic action; rather, they increase the efficiency of endogenous GABA in its postsynaptic effects. According to the model, when benzodiazepine receptors are occupied, an allosteric change in the GABA receptor complex occurs that favors increased binding of GABA to its own receptor. The ultimate result may be an increased GABA-mimetic effect on postsynaptic membrane function.

However, glycine is not a mere neurotransmitter. It is a ubiquitous amino acid, present in various cells and mediating various physiological functions, as well as being a constituent of diet proteins from plant origin (Peas, Polished rice, whole oats, etc..). It is an important constituent of bacterial cell wall (Salton and Tomasz, 1974).

Glycine is the main amino acid in collagen (3630 umoles/g protein) (Bowman and Rand, 1980). It forms an important amino acid constituent of other polypeptides e.g. insulin; Lysozyme; Histone; etc..)