INTRODUCTION

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Harris et al., (1985) defined diabetes as a state of chronic hyperglycemia (i.e., the state of having an excessive concentration of glucose in the blood), which may result from many environmental and genetic factors, often acting jointly. The term diabetes mellitus covers a constellation of metabolic abnormalities characterized by hyperglycemia and disordered carbohydrate, lipid and protein metabolism. These abnormalities may lead to serious late complications *(Edwards and Weston, 1995).*

The primary regulator of glucose concentration in the blood is insulin (synthesized and secreted by beta (B) -cells of the islets of Langerhans in the pancreas), which is the only hormone that reduces blood glucose concentrations. The regulatory mechanisms include inhibition of glucose production and release from the liver, enhancement of glucose transport into peripheral cells and alterations in general cellular glucose metabolism *(Marshall et al., 1991).*

Diabetic symptoms may be acute such as polyuria, polydepsia, fatigue, blurred vision, weight loss, ketosis and skin infection *(Edwards and Weston, 1995)*; or involve long term vascular and neural complications *(Vaccaro, et al., 1984)*. Due to its acute and long term complications especially in elderly diabetes is a major cause of morbidity and mortality and its increase in prevalence in many populations around the world *(Edwards and Weston, 1995).*

Since diabetes is characterized by hyperglycemia, treatment has the goal of restoring blood glucose to normal levels to avoid acute symptoms

and to prevent the onset of late complications *(McCarthy et al., 1977)*. The daily blood glucose profile of diabetic person should be as close as possible to that of a non diabetic person, including the peaks and troughs induced by diet, exercise, stress and emotion *(McCarthy et al., 1977)*. Control and complications trial (conducted in insulin-dependent diabetes mellitus (IDDM) patients but also considered relevant to non insulin-dependent diabetes mellitus (NIDDM) have confirmed that normalizing blood glucose levels can reduce the incidence of diabetic late complications *(DeFronzo et al., 1985)*.

The management of patients with NIDDM is not straight forward because the patient population has heterogeneous manifestations of the disease. Hyperglycemia in these patients may be due to impairment of insulin secretion by the pancreatic B-cells, insulin resistance in peripheral and /or hepatic cells, and other factors, alone or in combination *(DeFronzo et al., 1985)*. Ideally, treatment would be tailored to the metabolic defects in each patient, but this is impracticable because of the difficulty of determining the precise contribution of the various mechanisms and because of the limited range of available treatment options. Treatment is possible with diet and exercise alone or with additional oral therapy if there is adequate insulin secretion. If not, parenteral insulin will be necessary *(McCarthy et al., 1977)*.

DIABETES AND NEUROTRANSMITTERS

Conditions causing a reduction of oxygen availability (anoxia) such as diabetes result in drastic changes in ion movements, levels of neurotrnsmitters *Yang et al (1998).*

The clinical manifestations of type-II diabetes often include nephropathy, retinopathy and neuropathies *(Vaccaro et al., 1984).* The pervasive nature of the disease within the peripheral nervous system is well characterized. It is only recently, however that consideration has been directed towards associating the central nervous system (C.N.S) with the detrimental effects of type-I diabetes. Some of the C.N.S. abnormalities associated with type II diabetes include neuronal atrophy axonal degeneration, glycogen accumulation, encephalomalacia, demyleinization and alterations in brain neurotransmitters.

Merali et al. (1988) said that despite the increased research efforts, the origins and mechanisms underlying these C.N.S. abnormalities remain largely enigmatic.

However K_{ATP} channels are present in the brain *(DeWeille and Lazdunski, 1989 and During et al., 1995)* where they have been initially identified with radiolabelled sulfonylureas *(Mourre et al., 1989).* Their level is high in substantia nigra (SN) globus pallidus but also in hippocampus, cerebellum and cortex *(Mourre et al., 1990).* They are located both pre and post synaptically *(Mourre et al., 1990).*

These K_{ATP} channels play an important role in connecting changes of extra cellular glucose levels in the brain to changes of the

neuotransmitter release ***(Lazdunski, 1996). Amoroso et al. (1990)*** found that both in vitro and in vivo, synaptic K_{ATP} channels are associated with GABA release in response to variations of the glucose levels in high glucose concentrations K_{ATP} channels ***(Lazdunski, 1996).***

A route for synthesis of catecholamines from tyrosine has been established in cerebral cortex and other tissues ***(McIlwain and Bachelard, 1985),*** and this stage is catalyzed by tyrosine hydroxylase enzyme ***(Muller et al, 1969),*** Fig. (1).

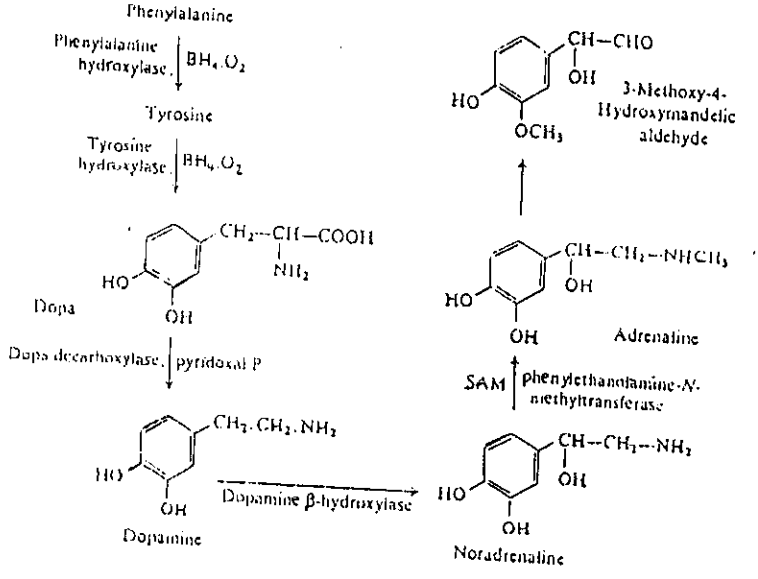


Fig (1): formation of catecholamines ***(McIlwain and Bachelard, 1985).***
BH4 : is a biopterin.
ASM: S- adenosylmethionine.

Tyrosine hydroxylase:

Its activity is highest in the caudate nucleus and substania nigra with very little detectable in the cerebral cortex. It is a rate-limiting step in the formation of norepinephrine and dopamine, and it is higher in dopamine containing nerve terminals than in noradrenergic terminals *(McIlwain and Bachelard, 1985).*

Dopa Decaroxylase:

The first catecholamine synthesized is dopamine, yielded by decarboxylation, through dopa decarboxylase enzyme *(McIlwain and Bachelard, 1985).*

Dopa decarboxylase enzyme is located in regions which coordinate the autonomic activities; in the reticular formation, hypothalamus, and certain parts of the thalamus. *(Pletcher, 1973).*

Dopamine B- hydroxylase :

Dopamine is converted to norepinephrine by dopamine β-hydroxylase, a copper containing enzyme, which demonstrated in brain cells.

McIlwain and Bachelard (1985) demonstrated the presence of dopamine β-hydroxylase enzyme in the caudate nucleus and hypothalamus in dog's brain, but in human brain it was highest in substantia nigra, and hypothalamus with little or non in the caudate nucleus or cerebral cortex.

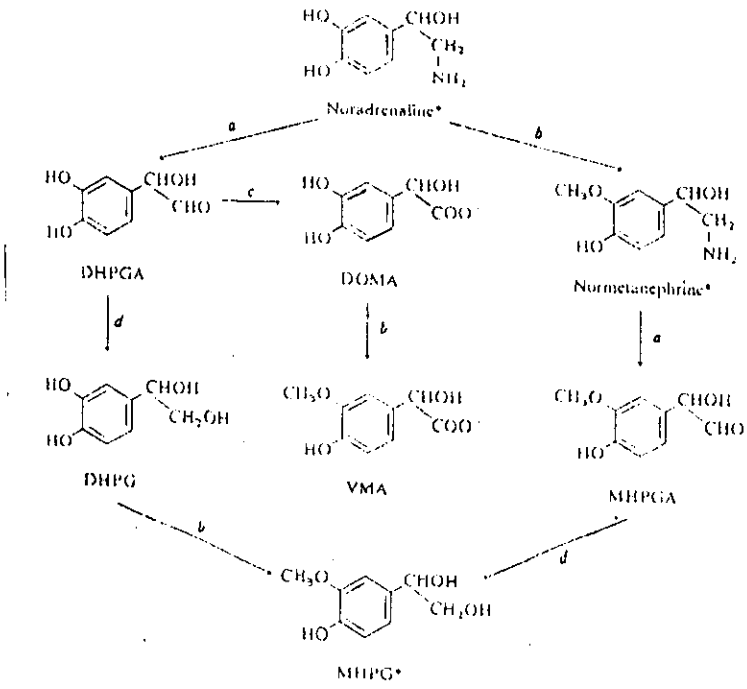


Fig. (2): Degradation of noradrenalin. a : monoamine oxidase, b: catecholamine0-methytransferace, C:aldehyde dehydrogenase, d: alcohol dehydrogenase

DHPGA : 3,4- dihydroxyphenlglycolaldehyd.

DHPG : 3,4 dihydroxyphenylglycol.

DOMA : 3,4 dihydroxymadelte.

VMA : vanillylamandeltat.

MHPGA : 3- methoxy 4- hydroxyphenylglycolaldehyde.

MHPG : 3- methoxy 4- hydroxyphenlglycol.

- May be excreted as the sulphate conjuage; MHPG, VMA and DOMA are formed form adrenaline also ***(McILwain and Bachelard, 1985).***

Noradrenaline is converted to adrenaline catalyzed by phynylethanol- amine-N-methyltransferase. ***(McILwain and Bachelard, 1985).*** Its activity is highest in the brain stem and in arcuate of the hypothalamus ***(Pletcher, 1973).***

Degradation of the Catecholamines:

McIlwain and Bachelard (1985) suggested for the inactivation of cerebral catecholamines a route already established in other organs, namely. Noradrendaline →3-0- methy l derivtive → 4-hydroxy-3-methoxy mandelic acid and glycol. Fig.(1,2).

Pletcher, (1972) added that the key anzymes involved are monoamine oxidase, catechoamine-0-methyl transferase, aldyde oxidase, and alcohol dehydrogenase. Hemovanillic acid is the major end product of dopamine metabolism, noradrenaline and adrenaline produce mainly 3-methoxy-4 dehydroxyphenlglycol., the brain whereas vanillylmandelic acid is the major product of their catabolism in the liver.

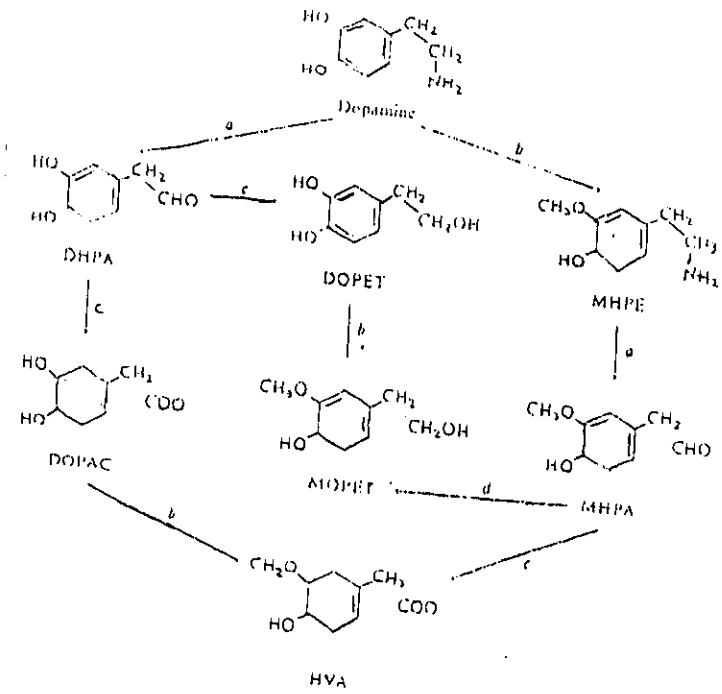


Fig. (3): Degradation of dopamine. A: monoamine oxidase, b: catecholamine0-methytrasferace, C: aldehyde dehydrogenase, d: alcholo dehydrogenase.

DHPA : 3,4- dighydroxyphenlglycolaldehyde.

DOPET : 3,4 – dihydroxyphenylethanol.

DOPAC : 3,- dihydroxymandelate.

MHPE :3- methoxy 4- hydroxyphenylethlamine.

MHPA :3- methoxy 4- hydroxyphenlglycolaldehyde.

MOPET : 3- methoxy 4- hydroxyphenylglycol.

HVA: homovanillate. (***McILwain and Bachelard, 1985).***

Vanillylmandelic acid is the major product of their catabolism in the liver.

Catechelamine-O-Methyltransferase: (COMT)

Feldman and Quienzer, (1984) detected two forms of the enzyme in human brain, and one form in rate brain.

The two forms are, the membrane bound and the soluble from. Membrane bound COMT has much higher affinity for catecholamines than does the soluble form ***(Kopin, 1985).*** He also added that membrane bound form of the enzyme is associated with non-neuronal tissues in the brain especially the endothelium and interface between blood and tissues. This suggests a role for COMT as an enzymatic barrier to free catecholamines diffusion.

The membrane bound COMT in the brain is located on the outer surface of postsynaptic membranes situated to inactivate neuronally released catecholamines ***(Kopin, 1985).*** The catabolic pathway for norepinephrine is slightly different in central nervous system. 3-4 dihydroxyphenylglycolaldycle (DOPGA) is reduced rather than oxidized to form 3-4 digydroxyphenlglycolal (DOPEG) which is then o-methylated by COMT to form 3 methoxy 4 hydroxypheniglycol (MHPG). This product may be further metabolized to MHPG- sulphate (MHPG.S) by the action of brian enzyme sulfotransferase ***(Feldman and Quienzer, 1984).***

Monamine Oxidase: (MAO)

Removal of amino group from various catecholomines is catalyzed in cerebral cortex and many tissues by monoamine oxidase ***(McILwain and Bachelard, 1985).***

There are at least two types of MAO, MAO_I and MAO_2, now designated MAO_A and MAO_B. MAO_B activity has been characterized according to inhibition by clorgyline, harmine, and harmaline. It is thermostable, inactivated by trypsin and has no immunological cross-reactivity with the liver enzyme. It is predominant in human hypothalamus, cerebral cortex and rat striatum, and acts preferentially on noradrenaline and serotonin. ***(Kopin, 1985)***

MAO_B was distinguished by its inhibition by deprenyl pargyline, and by tricyclic drugs, imiparmine and chlorpromazine. It act preferentially on tryptamine, benzylamine and β- phenylethylamines, is thermostable, trypsin stable and cross-reacts immunologically with liver enzymes ***(McIlwain and Bachelard, 1985).***

Although dopamine is acted on by both forms in many species, it seems to be oxidized mainly by the 'β' from in human brain and by 'A' form in rat brain ***(Kopin, 1985).***

The cerebral amine oxidase activity has been shown to be mitochondrial ***(McIlwain and Bachelard, 1985);*** and is affected by oestrogens and amphetamine. They also reported the presence of endogenous inhibitors of monoamine oxidase activity in rat liver and in human urine but has not been detected in cerebral extracts.

Dopamine:

Seeman, (1981) described 3 types of dopaminergic receptors, on the bases of sensitivity of the adenylate cyclase and on binding of agonist and antagonist drugs.

D_1 receptor are located on postsynaptic cells and activate dopamine sensitive adenylate cyclase, butaclamol and cis-flupenthixol are through to bind tightly to D1 receptor *(Sonsalla et al, 1986).*

Grebb and Reus (1984) demonstrated that presence of D_1 receptors in substania nigra, and located on the terminals of GABA ergic neurons origination within the striatum and globus pallidus.

D_2 receptors are located in presynaptic cells. Drugs as dihydroergocrptine, haloperidol and spiperone are considered to bind with a higher affinity to D_2 receptors which are adenylate cyclase insevsitive *(Sonsalla et al, 1986).* and are located in pituitary gland *(Grebb and Reus, 1984),* within striatum, ventral tegmental area (A-10 cell) region of the mesolimbic pathway *(Seeman, 1981).*

The synthesis and release of dopamine are regulated by end product control mechanism through presynaptic receptor D_2 *(Seeman, 1981).*

D_3 receptors which are located on presynaptic neurons, and their activation decreases the amount of dopamine synthesized in the presynaptic cells. They are found in the nigrostriatal and mesolimbic pathways but not in tuberoinfundibular and mesocertical pathways *(Grebb and Reus, 1984).*

Grebb and Reus (1984) stated that there are five recognized dopaminergic neuronal pathways in the central nervous system Fig. (4).

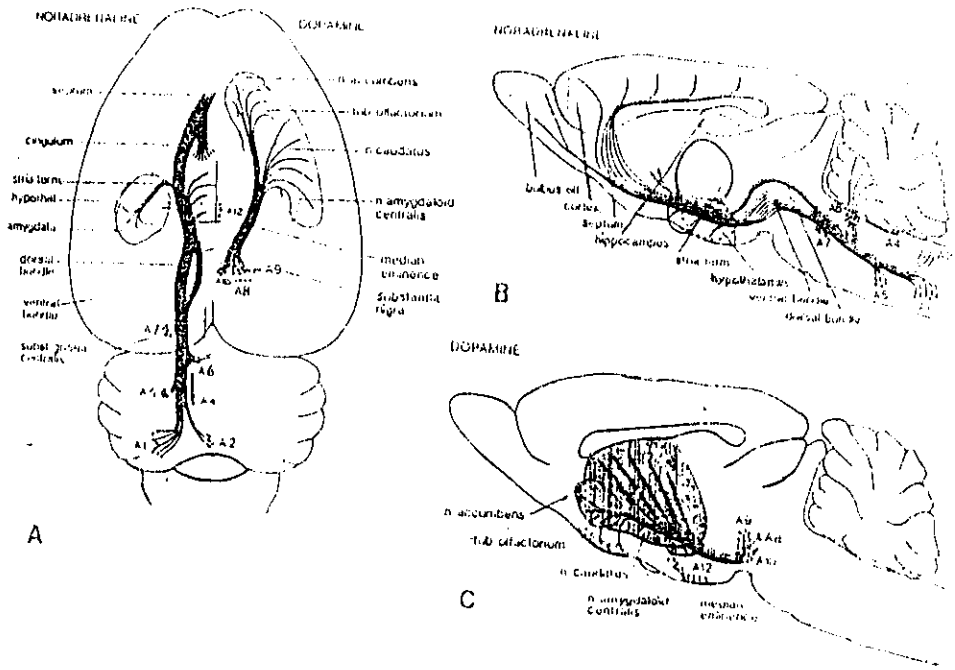


Fig (4) Neural pathways associated with transmission involving dopamine, and noradrenaline. The shaded parts indicate major areas containing nerve terminals A. Horizontal projection, B.C. saittal projeection ***(McIlwain and Bachelard, 1985).***

The nigrostriatal pathway has cell bodies of dopaminegic neurons in the substanita nirgra. These neurons project to the caudate nucleus and putmen of the corpus striatum. Normally, this pathway is involved in the initiation and coordination of muscle movement. Dopamine shares a dynamic balance with acetylcholine in this pathway ***(Grebb and Reus, 1984).***

In themesolimbic-mesocortical pathway, cell bodies of dopaminergic neurons exist in an area medial and superior to the substantia nigra. These neurons project to the limbic system and neocortex.

The tuberoinfundibular (tuberohypophyseal, hypothalamic-hypophyseal) pathway has cell bodies of dopaminergic neurons in the arcuate nuclei and periventricular area, these neurons project to posterior pituitary. This pathway regulates release of prolactin, from the pituitary. Dopamine itself is thought to act as prolactin-inhibiting hormone. *(Neal,1987).*

The medullary periventricular pathway has cell bodies of dopaminergic neurons in the motor nucleus of the vagus nerve and the nucleus tractus solitarii. The projection are not well defined, but this group of cells is thought to be involved in the control of food intake and perhaps in the phsiologic derangements causing eating disorders. ***(Grebb and Reus, 1984).*** They also added that a group of increatohypothalamic neurons from the dorsal and posterior hypothalamus to the dorsal anterior hypothalamus and the lateral septal nuclei. The role of these neurons is unknown.

Lozovsky et al. (1981) found that increased glucose and / or reduced insulin levels have been linked to hypofunctioing of the central dopaminergic (DA) system conversely, lesion of the of the dopamine neurons emanating from the substantia nigra has been noted to inflict marked impairment of pancreatic islet growth.

Amoroso et al. (1990) reported that high glucose levels inhbit K_{ATP} channels, depolarize substania nigra terminal, increase GABA release and thereby also extentensively inhibit dopaminergic neurones.

Merali et al 1988 found that 4 days deprivation of insulin in spontaneously diabetic rats did not induce marked alteration in the levels of dopamine, or its metabolites 3,4, dihydroxy phenylethanol in any of the brain regions except for the hippocampus, where a slight increase in dopamine level was observed. ***Kwok et al 1985*** found that in sponanously diabetic rats insulin deprived from time of diabetes onset (11-23 days), dopamine levels remained unchanged in several brain regions. However they reported decreased 3,4, dihydroxy phenylethanol levels at the striatum and the olfactory tubercle.

Bitar et al. (1986) using streptozotocin- diabetic rats, and ***Kolta et al 1986*** using alloxan- diabetic rats found no alteration in the brain levels of dopamine. *Lozovsky et al. (1981) and Saller (1984)* reported that hyperglycemia and / or diabetes induced reductions in dopaminergic neurotransmission can lead to the development of compansatory post synaptic receptor supersensitivity, as measured by increase in (^{3}H) spiperone binding.

Norepinephrine:

There are 5 types of noradrnergic receptors *(Langer 1981)* which show some differences in occurrence and distribution.

α_1 receptors are postsynaptic and do not activate adenylate cyclase enzyme when stimulated, they are condensed in olfactory bulb and hippocamppus *(Young and Kohar, 1979)* but most α_2 receptors are

presynaptic, activate adenylcyclase when stimulated they function to reduce norepinephrine release from pre-synaptic neurons *(Langer, 1981)* some α_2 receptors are postsynaptic in thalamus, hypothalamus and midbrain *(Kopin, 1985).*

β_1 receptors are postsynaptic and may be presynaptic they activate adenylcyclase when stimulated, ***Yeh and Woodward (1983)*** demonstrated β_1 adnenoceptor in cerebellum.

β_2 receptors are postsynaptic and activate adenylcyclase when stimulated, there is some evidence for presynaptic β_2 receptors Both types of beta receptors predominate at the end of locus ceruleus tract, ***(Grebb and Reus, 1984) Lefkowitz et al. (1991)*** added that β_3 receptor stimulation mediates lipolysis in adipose tissue.

Bitar et al 1986 reported that uncontrolled streptozotocin-induced iabetes was associated a time – related decrease in tyrosine hydroylaze (IH) activity and increased norepinephrin concentrations and decreased norepinephrin trunover rates in femal streptozottocin- diabetic rats. Also ***Merali et al. (1988)*** observed that increased hypothalamic and cortical levels of norepinephrin. ***Fushimi et al. (1984)*** reported also increased norepinephrin levels in the peripheral tissues (adrenal, skin, heart and kidney) in the streptozotocin diabetic rats. ***Fushimi et al. (1984)*** has been suggested that catecholamine accumalation and impairment of secretion may play some role in pathogensis of diabetic autoimmune neuropathy in man.

On the other hand norepinephrinebehave like a co-transmitter in intra-pancreatic adrenergic nevers and induce a dose dependent

inhibition of insulin release between 1 and 50 nm and induce a progressive increase in pancreatic out flow rate but it is strongly inhibited B- cells and was comparatively poor vasconstrictor *(Skglund et al 1991)*.

Serotonin (5-HT):

The route of formation of serotonin in the brain commences with the dietariy essential aminoacid, tryptophan. ***(McIlwain and Bachelard, 1985)***, Fig. (5).

From tryptophan, serotonin is synthesized by hydroxylase enzyme which is selectively inhibited by parachlorophenyl alanin. This is the rate limiting step which forms 5- hydroxytryptophan, that is convert to 5-hydroxytryptamine by decarboxylase enzyme. ***(McIlwain and Bachelard 1985)***. These later step is regulated by a feedback mechanism controlled by the level of 5-hydroxytrpytamine ***(Swonger and Constantine, 1976)***.

It is metabolised by monoamine oxidase (Preferentially by MAO-A) into unstable oxidative product acted upon by either aldhyde, or alcohol dehydrogenases to give 5-hydroxyindol acetic acid Fig. (5). The pineal body contains all enzymes required for synthesis of serotonin, and it has the highest concentration of this amine in the brain ***(Grebb and Reus, 1984)***.

High concentration of 5 HT (90% of body serotonin) is present in the entreochromaffin cells of gastro-intestinal tract, ***(Dugleish et al., 1953)*** another 8% in blood platelets, and only 2% within central nervous system ***(Twarog and Page 1953)***.

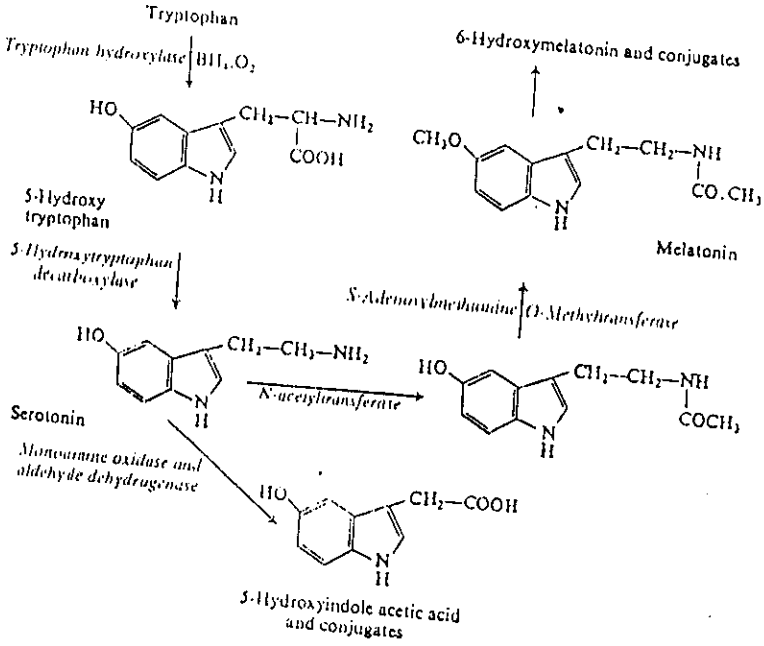


Fig. (5): Indolealkylamine metabolism in the brain. Formation of serotonin from typtophan, and its subsequent inactivation. ***(Chase and Walters, 1976).***

In the central nervous system, cerebral serotonin is stable postmortem and is highly localized, the cerebral cortex contaning less than 10^{-10} mol/gm with higher concentration in the midbrain pons, hypothalamus, and substantia nigera ***(McIlwain and Bachelared, 1985).***

Aghajanian et al., (1990) identified 3 types of serotonergic receptors, 5 HT1, require guanosine triphosphate for activity, and activate adenylate cyclas.

Fayol et al.(1988) reported that 5 HT binding sites of 5 HT type are heterogeneous and comprised several subtypes. 5HT (1A), 5HT (1B) and 5HT (1C).

5HT (1B) predominantly present in the basal ganglia of the rat and mouse brain *(Middlemiss and Huston, 1990)* they also added that 5HT (1B) activation inhibit transmitter release.

5HT (1A) mediates inhibitory effects on raphe neurons and hippocampal pyramidal cells, and 5HT (1C) receptors activate phospholipase C and thus increase intracellular concentration of calcium. *(Bloom, 1991)*

Aghajanian et al. (1990) demonstrated that 5HT-1 linked to the opening of K channels via pertussis-Toxin sensitive G protein, 5HT-2 modulated by activation of a second mesenger system, and 5 HT-3 directly interact with K channel rather than through coupling with a G protein or a second messenger.

Serotoninergic neurons are concentrated in the area of the median and the dorsal raphe nuclei, caudal locus ceruleus, area postrema, and interpeduncular area. *(Grebb and Reurs, 1984).*

The release of serotonin in the ascending sertoninergic system is controlled by γaminobutyric acid, it was found that GABA inhibits the

release of endogenous serotonin form frontal contex, *(Auerbach and Lipton 1985)*. Thus effect was mediated through GABA receptors *(Gray et al., 1986)*.

The effect of diabetes on serum and brain concetrations of serotonine was studied by ***Sumiyoshi et al 1997*** and found that the streptozotocin induced long lasting hyperglycemia is associated with a decrease in cerebral concentrations of serotonine and with an accompanying increase in the maximum number of 5-HT (1A) and 5-HT(2A) receptors in the brain and this may play a role in diabetes related behavioral abnormalities. On the other hand ***Thorre et al 1997*** reported that streptozotocin induced diabetes reduces serotonine synthesis and metabolism. ***Martin et al 1995*** study the effect of streptozotocin induced diabetes on the level serotonine in the peripheral tissue of rat and found that whole blood 5 serotonine HT levels decreased about 50% in diabetic rats then recovered their proper levels after one week of insulin therapy.

Webster et al. (1990) used image analysis and double labelling inmuno histochemistry on mesentric veins, and found that significant reductions in the denisty of nerve plexuses staining for 5 serotonine HT and tyrosine hydroxylase in vessles from diabetic rats.

Gamma amino buytric acid (GABA):

Gamma aminobutyric acid (GABA) appears to be a major inhibitory neurotransmitter in the mammalian central nervous system *(Passmore and Robson, 1980)*.

The metabolic pathway involving GABA is known as GABA shunt because it occurs as side trip of Kreb's cycle ***(Chase and Walters, 1976).*** The GABA shunt begins and ends with intremediates of Kreb's cycle.

GABA is formed from glutamic acid by an enzyme gulatmic acid decarbxylase (GAD) and is metablolized by gamma aminobutyric acid trasaminase (GABA-T) into succinic semialdhyde (SSA) ***(Grebb and Reus, 1984).*** The brain also contains dehydrogenase which catalysis the oxidation of succinic semialdhyde to suiccinc acid, which may enter tricarboxylic acid cycle. ***(Passmore and Robson, 1980),*** Fig. (6).

GAD is mainly localized in the presynapitc terminals. Uptake into presynaptic terminals or into surrounding postsynaptic neurons. Glia is the major way of removing GABA from its active site ***(Van Kammen, 1977).*** ***GABA-T*** is licalized in the mitochonctria of the postsynaptic neurons and the extraneuronal intracellular sites. ***(Cooper et al. 1982).***

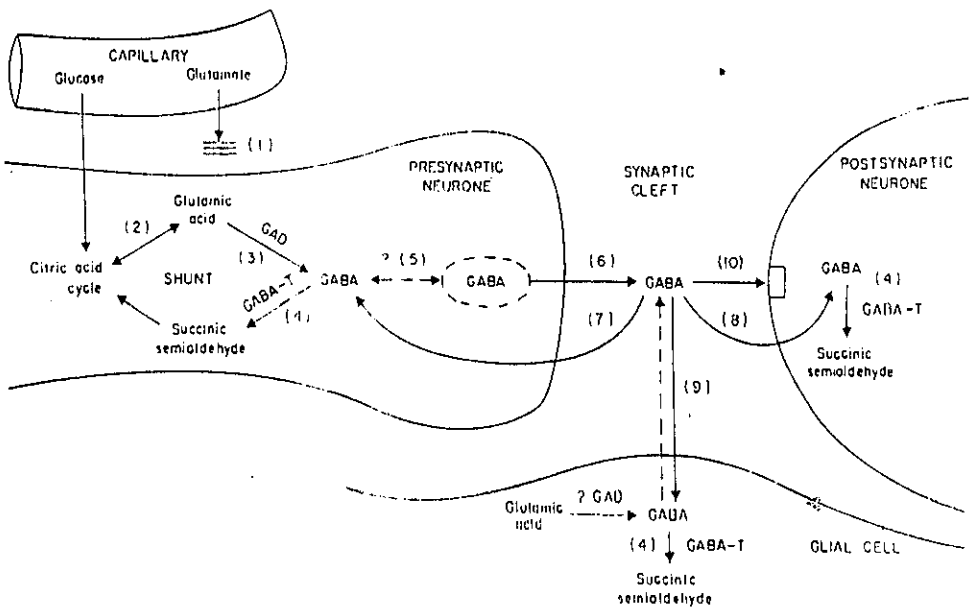


Fig. (6): GABA shunt and potential sites at which drugs may act to influence GABA-mediated synaptic function in the mammalian CNS: (1) blood-barrier; (2) precursor availability; (3) biosynthesis; (4) catabolism; (5) intraneuronal storage; (6) release; (7) uptake into presynptic terminals; (8) uptake into postsynaptic; neurones; (9) uptake into presynaptic glia; (10) receptor interactions *(Chase & Walters 1976).*

Pyridoxal phosphate serves as cofactor for GAD and GABA-T, and the concentration of GABA in brain tissues is probably controlled by GAD rather than by GABS-T ***(Passmore and Robson, 1980)***. They also added that monoamine oxidase inhibitors such as phenelzine and

phenylpropylhydrazine reduce GABA-T activity and raise GABA concentrations.

High uptakes of GABA by glial cells in the central nervous system might be an important mechanism for removing extracellular GABA after its release from inhibitory nerve terminals, GABA taken up in this way would be rapidly degraded by GABA-T. GABA-T activity if high in glial cells in both retina and brain ***(Van Kammen, 1977).***

GABA is widely distributed in inhibitory neurons, but is concentrated in the substania nigra, Globus pallidus, hypothalamus, and cerebellar and cerebral cortices ***(Passmore and Robson, 1980).***

It is the nerurotransmitter for the cerebellar Prukinje cells, which project to the vestibular and cerebellar nuclei. These is also a tract of cells containing GABA running from the corpus striatum to the substantia nigra that may interact in a complex relationship with the endogenous opioid system ***(Grebb and Reus, 1984).***

DiMicco and Gale, (1979) reported the role of GABA in specific brain regions for control of food intake, motor activity, synthesis of various transmitter and release of pituitary hormones.

There are probably at least 2 different types of GABA receptors in the central nervous system, GABA-A and GABA-B ***(Raiteri and Bonanno, 1987).***

GABA-A receptors, are postsynaptic receptors ***(Parfitt et al., 1990),*** and have been demostrated on dopaminergic nerve endings within

the striatum, cerebellum. *(Wood and Altar, 1988),* on dopaminergic cell bodies in the substancia nigra, on nerve endings of afferents to the substantia nirgr, frontal cortex, and hippocampus. *(Parfitt et al. 1990).*

GABA-B receptors may exist at pre-and postsynaptic sites depending on the brain region. *(Bowery et al., 1983),* they also demostrated their prescence in peripheral tissues, namely in intestinal muscle, vas deference, vascular muscle and basilar artery. Their distribution within the central nervous system was reported by *(Wood and Alter, 1988, Parfitt et al. 1990),* within hippocampus, substantia nigra, striatum, and cerebellum of the rat.

Parfitt et al (1990) demostrated that activation of GABA-B receptor diminished evoked GABA release and reducing the postsynaptic response.

However K_{ATP} channels are present in the brain *(DeWeille and Lazdunski, 1989; During et al 1995).*Where they have been initially identified with radiolabelled sulfonylureas *(Mourre et al 1989). Mourre et al (1990)* reported that their level is high in substantia nigra (SN), globus pallidus but also in hippocampus, crebellum and cortex they are located both pre and post synaptically *(Mourre et al 1990)* .

These K_{ATP} channels play an important role in connecting changes of extracellular glucose levels in the brain to changes of the neurotransmitters release *(Lazdunski 1996). Amoroso et al 1990* found that both in vitro and in vivo synaptic K_{ATP} channels are associated with GABA release in response to variations of the glucose.levels *(Lazdunski 1996)* reported that in high glucose concentrations K_{ATP} channels close as

in pancreatic B-cell and this closure ultimately leads to GABA release (as it leads to insulin release in B- cells). In hypoglycemic or hypoxic situation K_{ATP} channels tend to open and decrease GABA release.

SULFONYLUREAS

Two main classes of oral hypoglycemic agents used extensively are the sulfonylurea derivatives and less frequently prescribed biguanides. The sulfonylureas increase endogenous insulin secretion in the presence of glucose or certain amino acids, and may potentiate the action of insulin. ***(Yarborough and Steil, 1989).***

The sulfonylurea oral hypoglycemic agents are widely used and effective in the management of predomminantly insulin resistant diabetes in the early stages during which the secretory capacity for insulin is relatively well preserved ***(Lebavitz, and Passmantier, 1990).***

Sulfonylureas initially act by further increasing insulin secretion thus counteracting the insulin resistance correction of the hyperglycemia in turn further improves the responsiveness of the islet cell to glucose, and may account for much of the lessening of insulin resistance ***(Boyd et al 1991).***

Chemistry of sulfonylurea:

The sulfonylurea hypoglycemic agents are all having the basic structure R_1- [Benzene Ring]-SO_2NH CONH $-R_2$.

The main effects and mechanism of action are essentially the same for all the variants containing this central structure ***(Lebovitz and Passmantier 1990).***

Structure of sulfonylurea :

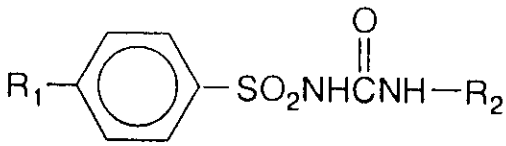


Fig (7): Structure sulfonylurea Oral Hypoglycemic Agents ***(Lebovitz and passmanitier 1990)***

Indication for use of sulfonylurea:

The sole indication for sulfonyurea treatment is NIDDM ***(Waldhausl 1996)*** physical training added to dietary control are essential since both lessen insulin resistance and can provide a more normal pattern of oxidative glucose disposal and reduce excessive hepatic gluconeogensis.

Yarborough and Steil (1989) reported that patients likely to benefit from the sulfonylurea drugs those who are obese rather than lean, their fasting plasma glucose concentrations under 180 mg/dl and no evidence of predominant insulin defiency such as low insulin / glucose ratio. Secondary failure in those responding initially occurs in approximately 5 – 10% per year of uncharacterized patients with ultimate failure in about 50% not obese patients, those with sever fasting hyperglycemia > 150 mg/dl and those with evidence of slowly developing autoimmune diabetes ***(Lebovitz and Passmaniter, 1990)***.

The major adverse effects of this group are weight gain with increasing insulin resistance, hypoglycemic coma particularly by interactions with other drugs and rare but serious toxic or allergic reaction *(Hansen and Christensen, 1977).*

Mechanisms of the hypoglycaemic effects of sulfonylureas:

Dean and Muthews (1968) found that these drugs depolarize the pancreatic B-cell membrane and induce electrical activity. Subsequent flux studies indicated that this depolarization resulted from a decrease in the K-permeability of the B-cell plasma membrane ***(Henquin, 1980). Sturgess et al. (1985)*** had revealed that sulfonylureas act as specific blockers of a particular class of K-channels in the B-cell, known as ATP-sensitive or K-ATP channels. In addition to B-cells, K-ATP channels have been identified in skeletal muscle, smooth muscle, pancreatic B-cells, kidney and neurones *(Aschcroft, 1988 & 1990)*. Their most characteristic property is that they are closed by an increase in the intracellular ATP concentration and thus they couple the metabolism of the cell to its electrical activity. As a consequence they have an important functions in many tissues under both physiological and pathophysiological conditions.

Sulfonylurea receptor:

For many years there was considerable debate about whether the K-ATP channel and the sulfonylurea receptor are the same protein, or whether the sulfonyluea receptor is a separate protein which interacts with the K-ATP channel. It is now clear that the B-cell K-ATP channel is a complex of (at least) two different proteins ***(Inagaki et al., 1995 and Sakura et al., 1995)*** (Fig. 2). One of these proteins (kir 6.2) is a K-channel subunit and it is likely that four Kir6.2 subunits come together to

form the channel pore. The other protein, the B-cell sulfonylurea receptor (SURI), acts as a regulator of channel activitiy conferring sulfonylurea sensitivity and possibly also ATP-sensitivity. The number of SUR1 subunits which contribute to the fully functional K-ATP channel is currently unknown *(Aschroft, 1996)*.

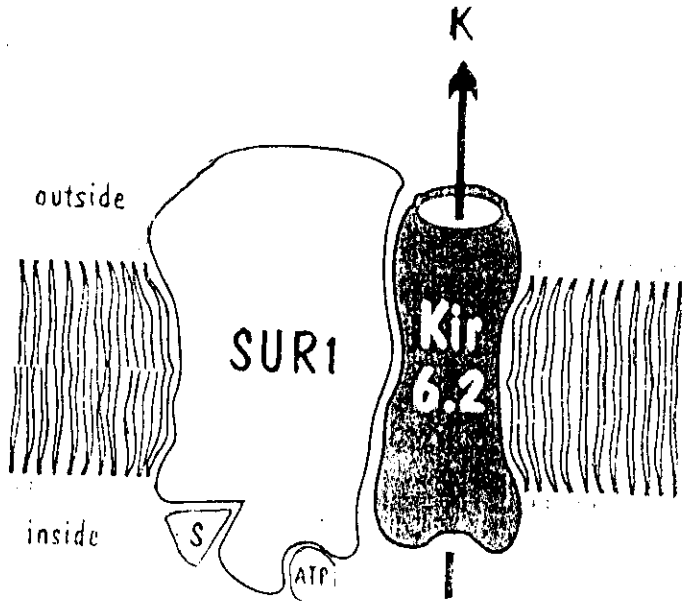


Figure (8): Sulfonlyurea receptor.

Aschroft (1990)

Aschcroft (1990) reported that the pancreatic B-cell, the K-ATP channel plays a key role in the regulation of insulin secretion in response to both nutrient secretagogues and to sulfonylurea. The primary physiolgical stimulus for insulin secretion is a rise in the blood glucose concentration. *Aschcroft (1996)* reported that glucose enters the B-cell and is metabolised, both by glycolysis and within the mitochondria, to ATP. *Kramer et al. (1996)* found that elevation of the intracellular ATP concentration causes the closure of K-ATP channels in the B-cell plasma membrane, thus reducing the membrane K-permeability and producing depolarization as a result, voltage-dependent Ca2+ channels are opened,

which increases Ca2+ influx into the cell and the subsequent rise in intracellular Ca2+ triggers exocytosis of insulin granule *(Kramer et al., 1996).*

Sulfonylurea also mediate inhibition of K_{ATP} channel activity and thereby initiate the same chain of events, culminating in insulin release. In contrast *Aschroft (1996)* reported that in contrast, the drug diazoxide opens K_{ATP} channels thereby hyperpolarizing the B-cell and inhibiting insulin release. *Lazdunski (1996).*

Extrapancreatic Activity:

Kramer et al. (1996) demonstrated that in short term therapy of NIDDM, sulfonylureas cause reduction of blood glucose predominanty via the stimulation of insulin release from pancreatic B-cells. *Muller and Geisen (1996)* observed that in long-term treatment of NIDDM patients with sulfonylureas their plasma insulin levels return to near-pretreatment levels without concomitant loss of hypoglycemic control and this is explained by-insulin independent activities of these drugs exerted on both pancreatic and extrapancreatic tissues *(Feldman and Lebovitz, 1971; Hosker et al., 1985 and Kolterman et al., 1994).* Possible mechanisms include inhibition of glucagon release, reduction of removal of insulin by the liver, facilitation of the transport of insulin across endothelial cells, increase of insulin sensitivity of peripheral target cells and direct stimulation of glucose utilization in muscle and fat tissues *(Muller and Geisen, 1995).* The major pathway for glucose utilization is the non-oxidative glucose metabolism with the two branches glycogen and lipid synthesis *(Muller and Geisen, 1995)* The rate of lipogenesis and glycogenesis in vivo is controlled by the glucose transport which is stimulated by glimepiride and glibenclamide through dephosphorylation

and activation of key enzymes of glucose transport and metabolism *(Muller and Geisen, 1996).*

Pharmacokinetics:

Various sulfonylureas differ in their duration of action, hepatic metabolism, bioactivity of metabolities, and route of excretion, *(Gerich 1985).* In addition, there are individual variation concerning their pharmacokinetics *(Lebovitz and Passmantier 1990).* Thus, close self-monitoring of the profile the blood glucose response is essential *(Gerich 1985).*

Gerich (1985) reported that there is much variation between individuals in the rate of absorption of sulfonylureas, and this may confound to the results of clinical trials. Because of slower absorption, tolbutamide and glipizide are best given one half-hour before a meal. A diet high in fiber may further delay absorption to some degree. *Yarborough and steil (1989)*

Glimepiride:

Glimepiride (Amaryl,) is a new second-generation sulfonylurea, developed to normalize the blood glucose profile of non-insulin dependent diabetic patients *(Robertson and Home et al., 1993).*

Chemical structure:

Its chemical structure 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea *(Kramer et al., 1995).*

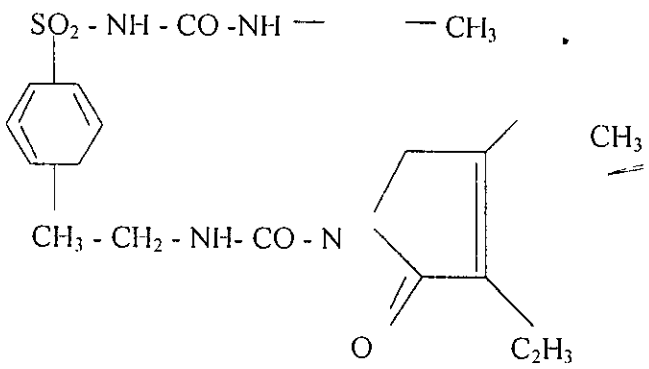


Fig. (9): Structural formula ***(Kramer et al., 1995)***

Glimepiride was approved by the Dutch Health Authorites First in June, 1995. The Fedral Drug Administration in the United States approved Glimepiride in November, 1995.

It has B-cytotropic effect similar to glibenclamide (***Kramer et al., 1995)*** with a rapid onset and long duration of action, excretion is not solely through the kidney, an effective miligram dose and good tolerability and lower insulin and C-peptide levels possibly due to less stimulation of insulin secretion and more pronounced extrapancreatic effect ***(Draeger, 1995).*** In normal man the hypoglycemic effect of oral glimepiride is apparent with doses of 1 mg and 1.5 mg the minimal effective dose is 0.6 mg and glimepiride is well tolerated in doses up to 9 mg ***(Tsumura and Draeger, 1995).***

Pharmacokinetic of glmepiride:

Glimepiride is absorbed rapidly and completely from the gastrointestinal tract after oral administration (absolute bioavailability 97-100%) ***(Badian, 1994).***

Studies with single oral dose in normal subjects and with multiple oral doses in patients with NIDDM have shown significant absorption of glimepiride within one hour after administrated and peak drug levels (C_{max}) at 2 to 3 hours. Glimepiride was given after meals, the rate of absorption (T_{max}) was slightly decreased, but the extent of absorption (AUC = area under the curve) was unaffected ***(Anon 1996)***.

After I.V. administration of 1 mg, glimepiride had a very low distribution volume (8.8 liters, which approximates albumin distribution space) and a low clearance (48 ml/min) ***(Levein and Bakar 1996)***.

Glimepiride has a low solubility (0.03 mg/l at pH 1.2, 7.3 mg/L at P.H 7.4 and octanol water partition about 100 at P.H 7.0; Pka 6.2), glimepiride and both its metabolites are highly protein bound, mainly to albumin, with unbound fractions of less than 1% (glimepiride) or 2-4% (M_1 and M_2) ***(Malerczyk et al., 1994)***.

The pharmacokinetics of the glimepiride were linear in a single dose study in normal subjects using doses of 1 mg to 8 mg /day and in a multiple dose study in patients using doses of 4 to 16 mg/day a slight deviation from linearity for C_{max} could be explained by the poor solubility of the drug and was not clinically important. There was no differences in pharmacokinetics between normal subjects and patients with NIDDM ***(Malerczyk et al., 1994)***. At steady state, mean peak concentrations of glimepiride (C_{max}) after 4 mg and 8 mg oral doses were approximately 300 ng/ml and 600 ng/ml respectively. Glimepiride did not accumulate in plasma with repeated oral dosing once or twice daily. After intravenous dosing in normal subjects, the volume of distribution was 8.8 liters (113 ml/kg), and the total body clearance was 47.8 ml/min. No relevant

pharmacokinetic difference between single and multiple dosing was observed. Glimepiride clearance, in particular was the same indicating that the elimination of the drug is not changed during longer treatment *(Levein and Bakar, 1996).*

Serum half-life of Glimepiride:

Mean half-life after a single dose of 2-8 mg glimepiride ranged from 5.2 to 7.2 hours. While the mean half-life after multiple doses of 4 or 8 mg was 7.8 and 8.8 hours, respectively *(Anon 1996)* It can be concluded that there is prolonged elimination of glimepiride most likely because of a gradual release from tissue binding, a finding which supports a dosing interval of 24 hours. However, since the concentrations observed during the terminal phase after the 16 mg dose were very low, they did not contribute to the production of the multiple dose-concentration profile. Therefore the disposition of glimepiride can be characterized by a half-life of 5 hours *(Malerczyk, 1994).*

Glimepiride is completely metabolized *(Mulerczyk, 1994)*. The major metabolites are the cyclohexyl hydroxy methyl derivative (M_1) and the carboxyl derivative (M_2). M_1 is further metabolized to M_2 the conversion of glimepiride to M_1 followed by further metabolism to M_2, is the major metabolic pathway. After oral administration, terminal half-life of M1 was 3-6 hours. M_1 and M_2 have no clinical role in glucose lowering *(Badian et al., 1996).*

Three healthy male volunteers received a single oral dose of ^{14}C-glimepiride. Recovery of radioactivity was almost complete (93%) after 48 hours, 58% of the dose was eliminated in urine and 35% in faces. Serum half-life of radioactivity during the primary phase of elimination

was 4.2 hours *(Badian et al., 1996)*. Thus, no accumulation of drug related material is expected during single daily administration. *(Anon 1996)* radiolabelled material was distributed between plasma and whole blood in a ratio of about 2:1, indicating no relevant up take by RBCs,. About 80-90% of the radioactivity excreted in urine was accounted for by the two metabolites the cyclohexyl hydroxymethyl derivative (M_1) and the carboxyl derivative (M_2) *(Levein and Bakar, 1996)*. .

M_1 is further metabolized to M_2; there was no parent drug *(Malerczk, 1994)*. The main metabolites identified in feces were also M1 (10-15% of the excreted amount) and M2 (45-50%). Less than 2% of radioactivity in the feces was from the parent drug, indicating almost complete bioconversion of glimepiride, most probably in the liver, in man (as in rat and monkey). Parent drug and both metabolites were identified in serum *(Malerczk, 1994)*. Other drug related radiolabelled material amounted to less than 5% since 61% of an I.V. dose was excreted via bile in the rat (*Levein and Bakar, 1996)*.

Lehr and Damm, (1990) reported that no parent compound was detected in 5 patients with biliary T-tube drainage after administration of 1 mg I.V. Glimepiride and M1 accounted for only about 2% of the dose. Because absolute bioavailability of glimepiride after oral administration was 100% and virtually no parent compound was found in feces, unabsorbed drug could not be the source of fecally excreted radioactivity. *(Badian 1994)* Instead it may be biliary excretion of conjugated metabolites or secretion via the gastrointestinal mucosa *(Lehr and Damm, 1990)*.

Langtry and Balfour (1998) found that no significant differences in glimepiride pharmacokinetics between younger and elderly NIDDM patients thus age alone dose not require adjustement in the dosage of glimepiride.

Patients with renal disease have a higher risk of developing hypoglycemia largely due to impairment of renal insulin elimination ***(Rabkin et al., 1970)*** or to decreased counter regulation of blood glucose ***(Balant, 1981)***. ***Rosenkranz (1996)*** found that the drug is more rapidly eliminated in terminal renal failure probably due to reduction in the plasma protein-bound fraction.

Pharmcodynamics of glimepiride

Glimepiride was compared with glibenclamide for its insulin secretion stimulating and B-cell membrane depolarizing activity as well as for its binding kinetics to B-cell membranes and for its B-cell membrane binding proteins. ***(Geisen 1988).*** Steady state, kinetic and competitive binding studies revealed a 3- to 4-fold lower binding affinity of glimepiride to isolated B-cell membranes and intact B-cells compared to glibenclamide ***(Kramer et al., 1996)***. Direct photoaffinity labeling of B-cell membrane proteins with the radiolabeled sulfonylureas identified a 65-kDa binding protein for glimepiride ***(Aguilar et al., 1990)*** and a 140-kDa binding protein for glibenclamide ***(Kramer et al., 1994)*** which may be the basis for the different binding characteristics of the two sulfonylureas. ***(Kramer et al., 1996)*** demonstrated a 3- to 4-fold lower depolarization activity of glimepiride compared to glibenclamide which correlates well with the lower binding affinity of glimepiride ***(Kamer et al., 1996)***.

Glimepiride has blood glucose decreasing activity as other sulfonylurea through inhibition of K-ATP channels at B-cell of the panceras and insulin release (***Kramer et al., 1996***), (Fig. 10).

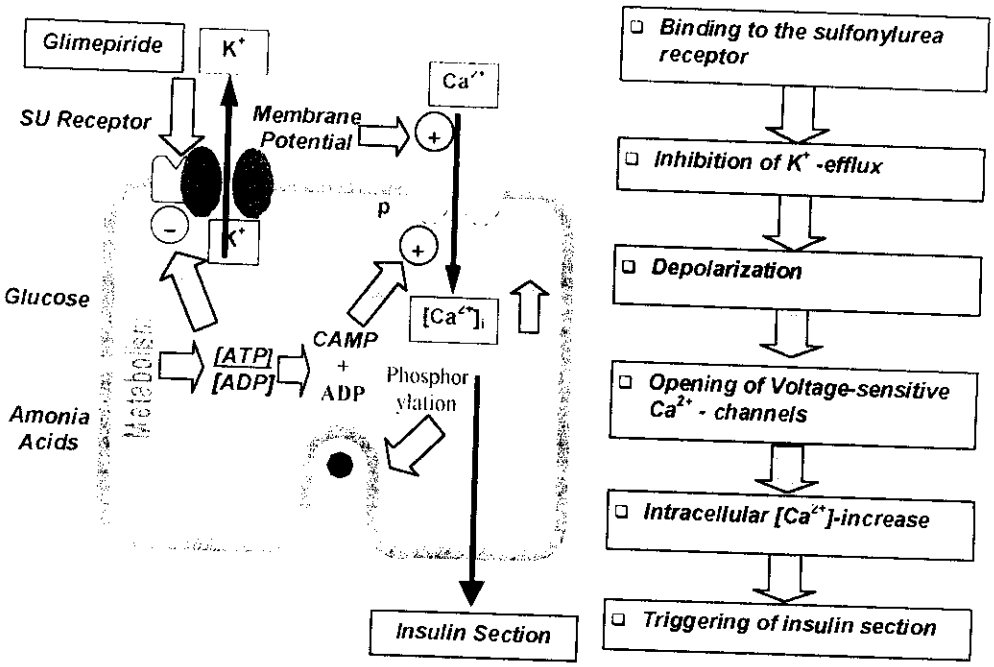


Fig. (10): Scheme for the molecular mechanism of insulin secretion from B-cells by sulfonylurea glimepiride drug (***Kramer et al., 1996***).

Muller and Geisen (1996) study the relation of the same dose of glimepiride and other sulfonylurea glibenclamide, glicalzide and glipizide with mean plasma insulin increase, mean blood glucose decrease PI/BG ratio in fasted dose in this study glimepiride has less insulin increase with more blood glucose decrease and so glimepiride has more insulin independent blood glucose decreasing activities than other sulfonylurea.

Other pharmacological properities of glimepiride:

Sulfonylurea in general have adverse cardiovascular effects in the therapeutic insulinotropic doses ***(Huupponen, 1987)***. This effect occurs also via ATP-dependent K^+-channels in cardiomyocytes ***(Noma and Shibasaki, 1985)*** and smooth muscle cells of blood vessels ***(Standen et al., 1989 and Silberberg and Van Breemen, 1990)***.

By inhibiting the ATP-dependent K+-channels in smooth muscle cells of coronary vessels sulfonylureas reduce coronary blood flow ***(Billman et al., 1993)*** and by inhibiting the ATP-dependent K+ channels in cardiomyocytes sulfonylureas delay myocardial repolarization time ***(Wilde and Aksnes, 1995)*** resulting in an antiarrythmic or proarrhythmic effect.

The evidence that glimepiride has less vascular activity than conventional sulfonylureas is derived from the following in vivo study In the anaesthetized hypoxic-shock dog, glibenclamide in a dose of 0.15 mg/kg i.v. and glipizide in a dose of 1.5 mg/kg i.v. induced an immediate increase in blood pressure. Glimepiride in doses up to 0.45 mg/kg i.v., had no effect ***(Landry and Oliver, 1992).***

Glimepiride has less influence than glibenclamide on the cardiomyocyte is derived from the following in vitro study done by ***(Ballangi et al., 1992).*** In the isolated rabbit heart, glibenclamide at concentrations of 1-100 umol/l dose-dependently elevated the electrical threshold, conduction time and effective refractory period and dose-dependently decreased the automaticity. Glimepiride, in the same concentrations, had only mild effects or none at all.

Anti-atherogenic effect of glimepiride:

Glimepiride inhibited thrombin stimulated increase of intracellular Ca^{2+} in platelets from human volunteers at concentrations of 20-40 uM *(Ozaki et al., 1992).*

Colwell (1990) demonstrated that glimepiride inhibited selectively the cyclooxygenase pathway at concentrations up to 40 uM, whereas glibenclamide inhibited both the cycylooxygenase and 12-lipooxygenase pathways glicalizde has no effect on either cyclooxygenase or 12-lipooxygenase.

Ozaki et al. (1992) reported that glimepiride inhibits platelet aggregation, a fact which suggests a preventive effect on the development of late diabetic microvascular complications in patients. This effect is more pronounced than the effect of glicalizde and more specific than the effect of glibenclamide.

Sulfonylurea Glimepiride and Neurotransmitters:

ATP-sensitive potassium channels are found in a number of different tissues where they play distinct physiological functions *(Lazdunsk, 1996).* These channeles are an important class of ionic channels linking the bioenergetic metabolism to membran exitability. They are particulary well represented in the brain *(Aschcroft, 1990).*

K_{ATP} channels are inhbited by antidiabetic sulfonylurea where they have been identified with radiolabelled sulfonyluneas *(Mourre et al 1989).*

In the presence of, sulfonylunrea K_{ATP} channeles close and this closure leads to GABA release *Amoroso et al. (1990)* reported that antidiabetic sulfonylurea inhibit K_{ATP} channels, depolarize substania nigra terminals, increase GABA release and thereby also extensively inhibit dopaminengic neurones.

VITAMIN E (ALPHA – TOCOPHEROL)

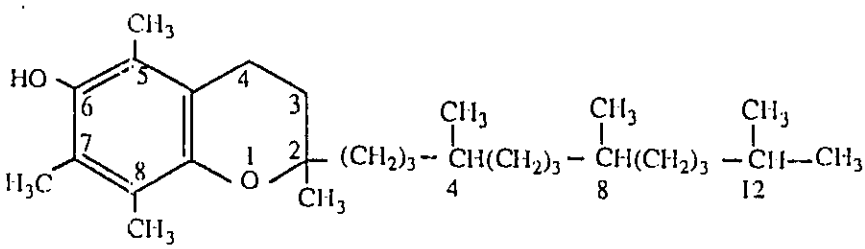
Introduction:

In 1922, Evans and Bishop discovered vitamin E by isolation of a substance that helped rats to produce robust offspring. The substance was subsequently indentified as a fat-soluble vitamin. The term tocopherol was proposed by Evans from Greek noun "tocos" which means childbirth or offspring and "phero" that means to bear or bring forth and "ol" from alcohol.

The letter E was chosen because the antisterility vitamin was the fifth to be recognized, coming after vitamin A (antiscorbutic), vitamin D (antirekitic).

Chemistry of vitamin E:

Pure vitamin E was isolated by *Evans and Emersons (1936)* from unsaponifiable fraction of wheat germ oil. It was identified chemically by *Fernhollz (1938)* and synthesized by *Karrer et al. (1938).*



α– tocopherol

Fig. (11): α - tocopherol *Karrer et al. (1938)*

Alpha-tocopherol (5,7,8, trimethyl tocol) is considered to be most important tocopherol since it comprises about 90% of tocopherol in animal tissues *(Olsen, 1977).*

Although alpha-tocopherol is the active compound most often designated as vitamin E, there are other seven naturally exiting tocopherols which are designated by the number and position of methyl groups on either tocol or toctrienol complex. The eight naturally occurring tocopherols are α-, β-,γ- and δ- tocopherols and α-, β-,γ- and δ-tocotrienols *(Ayres, 1984).*

The vitamin E is a light yellow viscous oil melting at 2.3 to 3.5 °C and soluble in fat solvents.

The phosphate and acetate esters of alpha-tocopherol are most frequently used synthetic forms *(Ayres, 1984).*

Mode of action of vitamin E:

The mode of action of vitamin E at the molecular level is not known with certainty. At present, there are three hypothesis, the antioxidant hypothesis, the respiratory chain hypothesis and the genetic regulation hypothesis *(Olsen, 1977).*

The antioxidant hypothesis postulates that tocopherol is a physiological antioxidant which is designed to protect polyunsaturated fatty acids and other easily oxidisable groups in tissues against the effect of oxygen. Because vitamin E acts as an antioxidant, thus it plays an important role in stabilizing the lipid of cell membranes, because lipid portion lipoprotein of membranes tends to combine with oxygen, and this

lipid peroxidation liberates free radicals of unpaired electrons which have a very destructive effect upon cell membranes and the membrane of intracellular organelles *(Ayres, 1984).* Since vitamin E protect cell membranes, it should be of value in treating autoimmune diseases *(Ayres and Mihan, 1978).*

The respiratory chain hypothesis postulates that tocopherol plays a specific role in electron by serving as a catalyst for respiration *(Nason and Lehman, 1965),* or by regulating specific enzyme and cofactor concentration in mitochondria.

It has been suggested that vitamin E may be interconnected with Co-enzyme Q function in the respiratory chain because of the structure closeness of these two types of compounds and their similar biologic activities *(Roels, 1967).* A decline in respiratory capacity of mitochondria from animals undergoing hepatic necrosis from selenium or vitamin E deficiency is partially reversed by antioxidant in vitro, and is associated with reduced lipoyldehdrogenase and NADH oxidase *(Schwarz and Baumgartner, 1970).*

The genetic regulation hypothesis postulates that tocopherol regulates in some way the transfer of genetic information from the chromosome to the whole cell. This implies that vitamin E regulates the synthesis of specific proteins and enzymes required in the differentiation or adaptation of given tissues. This chief evidence for this hypothesis is that some of the vitamin E deficiency syndrome are related to genetic diseases that are clearly the result of abnormal genes (muscular dystrophy, habitual abortion). DNA and RNA turnover are altered in dystrophic muscle of vitamin E deficient rabbits *(Dinning, 1955).* Lastly,

the contractile protein, myosin, from vitamin E deficeint dystrophic rabbits is altered in its primary structure to a more fetal type *(Lobley et al., 1971).*

Metabolism and biological active forms of vitamin E:

There are eight naturally occurring forms of vitamin E α-tocopherol possesses the greatest biological activity, probably because it is more efficiently retained by the body ***(Lobely et al., 1971).*** A metabolite of ^{14}C-d-α- tocopherol, which was chromatographically separable from α- tocopheronic acid and α- tocopherol quinone, was observed in liver extracts from rats and pigs ***(Lucy, 1972).*** The metabolite was a mixture of a dimer and trimer of vitamin E and had no biological activity ***(Green, 1970).*** The mixture which appears to be the endproduct of α- tocopherol oxidation in vivo could be produced in vitro by incubation of α- tocopherol with methyllinoleate or with methyl linoleate hydroperoxide ***(Lobley et al., 1971).*** These results point out the analogy between the oxidation products of vitamin E formed in vivo with those formed in the presence of auto-oxidizing fatty acids or their peroxides in vitro systems. This implies that the products formed in vivo could be the result of reactions between vitamin E and fatty acid – free radicals or peroxides.

Vitamin E function as a biological antioxidant:

Tappel (1970) has reviewed the evidence for in vivo lipid peroxidation and holds the view that the major biological function of vitamin E due to its antioxidant properties can be described as radical chain-breaking in its inhibition of lipid peroxidation. He describes the chemistry of lipid peroxidation, free-radical formation, and subsequent

damage to proteins, enzymes, and membrane systems. The lipofuscin pigments which accumulate in animal tissues with increasing age have been characterized as lipid-protein complexes, presumably derived from lipid peroxidation of polyunsaturated lipids of subcellular membranes ***(Leucy, 1972).*** These pigments can be detected by characteristic fluorescence spectra and increase in amount when mitochondria, microsomes, and lysosomes are incubated in vitro in the presence of oxygen ***(Green, 1972).*** By the same methods, the pigments were shown to be present in greater amounts in testes of mice raised on a normal diet without supplemental antioxidants as compared to animals raised on a supplemental diet ***(Tapple, 1970).*** Accumulation of these pigments in the normal aging process was discussed in relation to vitamin E and synthetic antioxidant supplementation ***(Tappel, 1970). Lucy, (1972)*** also favored the antioxidant role of vitamin E, and further suggested that lipid peroxidation is not a random autocatalytic process but results form the highly localized formation of free radicals, which is part of the mechanism of action of certain enzymes.

These studies with vitamin E may be taken as evidence to confirm the hypothesis of ***Tapple (1970)*** that vitamin E reacts or functions as a chain-breaking antioxidant, thereby neutralizing free radicals and preventing peroxidation of lipids within the membranes. However, the fact that α- tocopherols needed for the complete protection of mitochondrial and microsomal membranes even when the diet contains sufficient of the synthetic antioxidant, ethoxyquin, to protect chicks against encephalomalacia, indicates, as proposed by ***Lucy (1972)*** that d-α- tocopherol appears to have the advantage over other tocopherols and organic antioxidants in having the best access and longest retention in the tissues. ***Green, (1972)*** showed that α- tocopherol is concentrated in

phospholipid, and found that the synthetic, organic antioxidant, diphenyl-p-phenyl- enediamine (DPPD) is distributed throughout the body fat, and that vitamin E is concentrated in mitochondria and microsomes.

Similar results were reported by ***(Lucy 1972),*** who suggested that "vitamin E stabilizes membranes by virtue of specific physiochemical interactions between its vital side chain and the fatty acyl chains of polyunsaturated phospholipids", particularly those derived from arachidonic acid. Interaction are proposed between the methyl groups of the phenyl chain of α- tocopherol and the cis double bonds of the fatty acids. Thus the methyl goup at C_4 of α- tocopherol can fit into a pocket provided by the cis double bond nearest the carboxyl group. The methyl group at C_8 of tocopherol is then register and can interact similarly with the third cis double bond".

Green (1972) suggests that the formation of such a complex may have three functional consequences (1) inhibition of peroxidative destruction of polyunsaturated fatty acids in cells and in cellular membranes occurring either in vivo or in vitro; (2) prevention of permeability of biological membranes containing relatively high levels of polyunsaturated fatty acids; and (3) possible prevention of degradation of the membrane phospholipids by membrane-bound phospholipases in vivo.

Vitamin E and carbohydrate metabolism:

Alfred et al. (1983) demonstrated large diminution of hepatic, muscular and cardiac glycogen in rats kept for one year on vitamin E free diet, and when those rats were treated with doses of vitamin E (200 mg daily), glycogen accumulated rapidly. ***Butturini 1950),*** confirmed the previous findings and demonstrated increased muscular, cardiac, and hepatic glycogen in vitamin E overfed guinea pigs. The rapid accumulation of glycogen, was attributed either to enhancement of glycogenesis or to decreased glycogenolysis by α- tocopherol which inhibits phosphoglucomutase *(Lewis et al., 1973).*

Alfred et al. (1983) found constant diminution of blood glucose when vitamin E was administered orally to healthy subjects. *(Butturini, 1950),* in his experiment on guinea pigs confirmed the above finding. The hypoglycemic action of vitamin E through enhancing the process of glycogenesis in muscle and liver might be either due to enhancing effect on the β-cells of the islets of langerhan's (i.e on insulin secretion) or due to an inhibitory effect of the insulin antagonists *(McMasters et al., 1967). Butturini, (1950)* Found that α- tocopherol did not alter the insulin requirement of alloxan diabetic dogs. This would lead to the conclusion that vitamin E probably demonstrated its action by a direct stimulatory effect on β-cells of the islets of langerhan's.

Vitamin E and diabetes:

An association between vitamin E and human diabetics has been reported by a few investigators. ***Darpy et al. (1949)*** found that human diabetics had higher concentrations of plasma tocopherol compared with normal subjects. This finding was confirmed by *McMasters et al., 1967.*

However, *(Lewis et al., 1973)* found in a more recent study that means plasma tocopherol levels of non obese diabetics were not significantly different from age-and sex-matched control subjects. On other hand, *Alfred et al., 1983,* found that administration of the antioxidant vitamin E to rats proior administration of either streptozotocin or alloxan provided protection against the diabetogenic effects of both these drugs.