INTRODUCTION
Suppose a man sees a black snake just in front of him, and that he is able to take suitable evasive action, his retroaction would involve changes in the activity of a large number of cells in his body. First and foremost, the presence of a black and a long object would be registered by the visual sensory cells in the eye, and these, in turn, would excite nerve cells leading to the brain via the optic nerve. A great deal of activity would then ensue in different types of nerve cells in brain and, in seconds, the nerve impulse would pass from the brain to the muscle of the legs and arms. The muscle cells would contract upon the nerve impulse reaching them so as to move the body and prevent the man from stepping on to the snake.

A chain of events of this type involves the activity of a group of cell types, namely, nerve cells, and sensory cells, which can be described as excitable cells. An excitable cell, according to Aidley (1971), is a cell which readily and rapidly responds to suitable stimuli, and in which the response includes a fairly rapid electrical change at the resting cell membrane.
Now, let us critically analyse the above definition of an excitable cell. The resting cell membrane is electrically negative inside compared with the outside by some tens of millivolts. This potential difference is known as the 'Resting Potential' of the membrane. The negative resting potential, $V_m$, of the membrane is determined by the ease with which ions can cross the membrane.

Now the question is, 'how is this charge distribution achieved in a nerve cell?' This is illustrated in a nerve cell by the active outward transport of Na\(^+\) concomitant with the inward transport of K\(^+\). This process involves an obligatory exchange of 2 K\(^+\) for 3 Na\(^+\) with the help of an electrogenic, Na\(^+\), K\(^+\) pump, which derives its energy from the energy released by the hydrolysis of ATP catalysed by Na\(^+\), K\(^+\) activated ATPase present in the membrane.

I IONIC BASIS OF ACTION POTENTIAL

Let us now visualise the 'rapid electrical changes' at the resting cell membrane, when it responds to a suitable stimuli. In the resting state, the membrane is predominantly permeable to K\(^+\), hence the resting membrane potential approximates $E_K$. Potassium is nearly in electrochemical equilibrium across the
resting membrane. Sodium, more concentrated outside the cell than inside, has an electrochemical gradient opposite to that of $K^+$, and an equilibrium potential of opposite polarity to that of potassium. The Na potential, $E_{Na}$, is positive whereas $E_K$ is negative (Fig. A). Because the sodium equilibrium potential is far from the resting potential, there is a large electrochemical potential ($V_m - E_{Na}$) acting on $Na^+$. This electromotive force is a substantial source of potential energy, which is released in some cells by stimuli that increase the permeability of the membrane so as to permit a transient inward sodium current (i.e. net influx of $Na^+$) through the membrane (Eckert and Randall, 1978).

The effect of temporary increase in the Na conductance $g_{Na}$, is illustrated in Fig. A. When the Na conductance in series with $E_{Na}$ increases, $Na^+$ will flow through the increased $Na^+$ conductance into the cell, adding positive charge to the inside surface of the cell membrane, until a new steady state potential is attained between $E_K$ and $E_{Na}$, and $K^+$ (which is now out of equilibrium) carries charges out of the cell at the same rate as $Na^+$ carries them in. When $g_{Na}$ is restored to its previous low level,
the membrane potential returns to its original value near \( E_K \). Thus, the membrane will undergo a 'depolarisation' in response to an increase in its sodium conductance.

This is precisely the ionic basis of an action potential, which involves changes in \( \text{Na}^+ \) and \( \text{K}^+ \) conductance. It should be noted that (i) the intracellular resting concentrations of \( \text{Na}^+ \) and \( \text{K}^+ \) are restored by active transport before cumulative ion fluxes produce significant changes in ion gradient. (ii) The 'metabolic' pumping of ions across the cell membranes does not directly take part in the production or recovery of an action potential, but serves to maintain the ionic concentration gradients required for the production of membrane currents.

II PROPAGATION OF ACTION POTENTIAL

In order to understand the way in which impulse propagates, it is necessary to recall that once the excited membrane becomes permeable to \( \text{Na}^+ \), this ion carries a momentary current into the excited region of an axon. This current spreads longitudinally along the inside of the axon and leaks transversely outward across the membrane. This electronic spread of local circuit current is due to the cable proper-
Fig. A (Top) Changes in the membrane potential, $V_m$, produced by changes in sodium conductance, $g_{Na}$ (bottom). Note the changes in the terms $(V_m - E_{Na})$ and $(V_m - E_K)$ produced by the change in $V_m$. Note also that $E_K$ and $E_{Na}$ remain constant throughout (Eckert and Randall, 1978).
ties of the axon (Fig. B). Thus, the influx of sodium produces the upstroke of the action potential, resulting in a current which spreads longitudinally both forward and backward within the axon.

As the membrane ahead of the impulse is depolarised to the firing levels by local current, the sodium conductance of that region increases, initiating the regenerative sequence that produces the all-or-none action potential. The newly excited region then generates the local circuit current that depolarises and thereby excites portion of the axon ahead of it. Thus, the local circuit current from each excited region depolarises and excites in turn the next region ahead of it. The signal is continuously boosted and maintained at full strength in this manner as it travels along the axon, until it reaches the presynaptic nerve terminal of the synapse.

III SYNAPTIC TRANSMISSION

The action potential from the presynaptic to the postsynaptic nerve terminal is transmitted by the help of chemical messengers called the neurotransmitters. The sequence of events from the presynaptic action potential to the postsynaptic action potential is outlined in Fig. C.
The action potential in a nerve (top) is accompanied by local circuit current flowing across the membrane as shown (below). The 'foot' is produced by outward current depolarising the membrane ahead of the active (Na⁺ entering) region. Outward current in the inactive region is carried across the membrane primarily by K⁺ (Eckert and Randall, 1978).

**Fig. B**
Nerve action potential

Ca\textsuperscript{++} influx

Exocytosis

Neurotransmitter-receptor interaction

Postsynaptic permeability

Postsynaptic action potential

Fig. C Sequence of events from presynaptic to postsynaptic action potential (Eckert and Randall, 1978)
IV CHARACTERISTICS OF CHEMICAL TRANSMISSION

The following cellular processes represent the general characteristics of chemical transmission:

1. The presence of a presynaptic transmitter.
2. The presence of a presynaptic biosynthetic pathway for the transmitter.
3. The presence of a presynaptic transmitter storage mechanism.
4. The presence of a specific transmitter release mechanism.
5. The presence of a specific postsynaptic receptors.
6. The presence of a specific mechanism to terminate transmitter action.

The general characteristics of synaptic transmission are schematically represented in Fig. D.

1. Presence of a Presynaptic Transmitter

A large number of neurotransmitters are known to exist in the body, namely acetylcholine, glutamate, glycine, dopamine, noradrenaline, and 5-hydroxytryptamine. In the present introductory remark on the 'presence of the neurotransmitter', as a prerequisite for a chemical transmission process, the catecholamines which represent a class of neurotransmitters would be highlighted.
Fig. D  Schematic representation of synaptic function
(Triggle and Triggle, 1976a)
Catecholamine as Neurotransmitters:

Norepinephrine fulfills several criteria of a neurotransmitter: (a) The amine is highly localised in nerve terminals in vesicles. (b) It can be synthesised and stored locally, and a very efficient mechanism exists for its inactivation at the synapse. (c) In brain, an indirect evidence for release following nerve stimulation has been obtained (Aghajanian and Bunney, 1973).

Similarly, dopamine also fulfills all the above criteria of a neurotransmitter. By combined neurophysiological and histochemical methods, dopaminergic neurons have been observed to be located mainly in the zona compacta (ZC) of the substantia nigra and adjacent ventral tegmental (VT) area (Aghajanian and Bunney, 1973).

Aghajanian and Bunney (1973) reported that soma of dopaminergic neurons appear to have DA receptors since they are responsive to the direct, microiontophoretic application of either DA, or the DA agonist, apomorphine. If the terminals of dopaminergic neurons also have such DA-receptors (i.e. the presynaptic receptors), then this might explain the postulated receptor-mediated feedback control of striatal tyrosine hydroxylase activity at dopaminergic synapse (Kehr et al., 1972).
2. Presynaptic Biosynthetic Pathways for Neurotransmitter Amines.

The biosynthetic pathway for catecholamines synthesis is presented as follows (Triggle and Triggle, 1976a):

Phenylalanine → Hydroxylase → Tyrosine → Hydroxylase → DOPA → L-Aromatic Amino Acid Decarboxylase → Dopamine → Dopamine β-hydroxylase → Norepinephrine → Phenylalanine N-methyltransferase → Epinephrine
The response of an effector neuron to the chemically mediated transmission of a nerve impulse is not confined to immediate effects, such as changes in ionic permeability of the cell membrane or changes in the intermediary metabolism. The response also involves more permanent effects, reflected by changes in the synthesis of macromolecular cell constituents (Thoenen et al., 1973).

The trans-synaptic regulation of catecholamine synthesis involves an increased synthesis of tyrosine hydroxylase in the terminal adrenergic neuron. The regulation seems to take place at the level of transcription (Thoenen et al., 1973).

The regulation of tyrosine hydroxylase (TH) activity during nerve stimulation occurs with feedback inhibition by a variety of catecholamines (Weiner et al. 1973). Dopamine is approximately twice as potent as norepinephrine (NE) as an inhibitor of tyrosine hydroxylase. However, since the quantity of NE in adrenergic neurons is considerably greater than that of dopamine, presumably the end-product feedback inhibition of TH activity is mediated primarily by NE (Weiner et al., 1973).
Goldstein et al. (1970) reported that ouabain increased catecholamine synthesis from tyrosine in rat brain cortex slices. These investigators suggested that ouabain may enhance catecholamine synthesis by blocking uptake of released catecholamine thus reducing end-product feedback inhibition.

It would probably be an oversimplification to suggest that TH is the only regulatory step or the rate limiting step in the biosynthesis of catecholamines. Besides this step, other mechanisms too participate in the regulation process. In the first place, the substrates and enzymes of this pathway do not have free access to one another, since dopamine β-hydroxylase is sequestered in chromaffin granules and adrenergic vesicles. Some of the dopamine, which is formed from DOPA is taken up into vesicles and converted into NE and some is deaminated by the intraneuronal enzyme, monoamine oxidase (Molinoff and Orcutt, 1973).

The capacity of the adrenergic neuron to β-hydroxylate dopamine is relatively higher compared to its capacity to 3-hydroxylate tyrosine (Molinoff and Orcutt, 1973). A number of factors may, however, combine to limit dopamine β-hydroxylase (DβH)
activity in vivo. In the first place, since DβH is sequestered in storage granules, dopamine must be transported into these granules before it can be β-hydroxylated. A second potential mechanism for regulating DβH activity in vivo may involve the endogenous inhibitor(s) (??) of this enzyme (Molinoff and Orcutt, 1973).

Another aspect of the regulation of DβH and TH concerns inhibition of the synthesis of these enzymes (Brimijoin and Molinoff, 1971). For nerve terminals, to influence events in cell bodies, would require some means of carrying a signal between the two regions of the neurons. One possible mechanism is the retrograde axonal transport (Brimijoin, 1973).

3. Storage of Neurotransmitters

Once the neurotransmitter amines are synthesised and their transmitter function ascribed, the question is as to where are these transmitter amines stored and how are they released?

In the early electron microscopic studies on nervous tissue, small electron lucent vesicles with a diameter of about 500 Å were recognised as the outstanding intracellular component of the pre-synaptic nerve terminal (De Robertis and Bennet, 1955).
They were termed as synaptic vesicles and ascribed a functional role as storage sites for the neurotransmitter, and as the possible morphological basis for quantal release of these substances at the synapse (Hokfelt, 1973).

Different Catecholamine Storage Compartments:

Grillo (1966) recognised at least two different types of granular vesicles often described as small (500 Å diameter) and large (1000 Å diameter) granular vesicles (SGV and LGV respectively), in addition to classical granular vesicles. Subcellular fractionation studies indicated the presence of an extra-vesicular compartment containing 'free catecholamine' (Iversen, 1967; von Euhler, 1971).

At least part of these soluble amines may be derived from a new amine storing compartment described by Tranzer (1972), consisting of 'tubular reticulum like structure'. These structures not only exist in the nerve terminals but also in the whole axon, and presumably in the whole neuron (Tranzer, 1973). It is suggested that the three different compartments storing amines are formed in the cell body and probably migrate to the axonal
terminals by means of neuroplasmic flow and finally released by exocytosis (Geffen and Livett, 1971).

4. Release of Catecholamines and its Regulation

It is well established that Ca$^{++}$ plays an essential role in the release of neurotransmitters (Kagayama and Douglas, 1974). Akerman and Nicholls (1983) suggested that a low cytosolic Ca$^{++}$ activity in nerve endings is maintained by active Ca$^{++}$ transport system in the plasma membrane and membranes of intracellular organelles. During the electrical excitation in the form of action potential, Ca$^{++}$ ions flow into the cell through Ca$^{++}$ channels and the consequent increase in cytosolic Ca$^{++}$ triggers transmitter release.

There are several evidences for the role of cytosolic Ca$^{++}$ in transmitter release: (1) Removal of Ca$^{++}$ inhibits transmission (Kagayama and Douglas, 1974); (2) Microinjection of Ca$^{++}$ into nerve terminals causes transmitter release in the absence of electrical impulse (Katz and Miledi, 1967); (3) An inward electrical current carried by Ca$^{++}$ ions occur in activated nerve terminals (Katz and Miledi, 1969); (4) An increase in the cytosolic Ca$^{++}$ activity occurs upon activation of nerve terminals (Llinas and Nicholson, 1975); (5) Transmitter liberation from isolated
nerve endings is activated by Ca\(^{++}\) ionophores such as A 23187 (Cotman et al., 1976).

Calmodulin - a major Ca\(^{++}\) binding protein - has recently been implicated to play an important role in the neurotransmitter release and synaptic function (Delorenzo, 1982). The calcium-calmodulin complex activates the Ca\(^{++}\)-calmodulin kinase activity (Delorenzo, 1981). The Ca\(^{++}\)-calmodulin kinase system uses the soluble neurotubulin as substrates (Burke and Delorenzo, 1981), which upon phosphorylation undergoes marked changes in the physicochemical properties, causing a temperature-dependent formation of non-random, insoluble, filamentous like structures. These structurally oriented phosphorylated neurotubulins orient the synaptic vesicles (storage granules) on the synaptic membrane so as to assist exocytosis and finally quantal release of the neurotransmitter.

5. **Receptors and Regulation of Catecholamine Release**

Haggendal (1973) proposed a hypothesis of a presynaptic regulation of catecholamine release through a negative feedback mechanism mediated by adrenergic \(\alpha\)-receptors located on the presynaptic membrane. Langer (1973) suggested that the post-
synaptic α-receptor that mediates the response of the effector organ should be referred to as α₁ while the presynaptic α-receptor that regulates the transmitter release should be called α₂ (Fig. E).

Involvement of β-receptor facilitating NE release was demonstrated by Langer (1973). Further control mechanism at the synaptic cleft are offered by inhibiting muscarinic receptors decreasing norepinephrine release, and the presynaptic nicotinic receptor which elicit NE release (Muscholl, 1973).

Transmitter monoamines once released by exocytosis from the presynaptic nerve terminals into the synaptic cleft actually elicit their biological response on the postsynaptic membrane via receptor proteins on the postsynaptic membrane which recognises them. Greengard et al. (1973) have investigated the possibility that cAMP formed in response to certain neurotransmitters may actually mediate the electrophysiological response of the postsynaptic membrane to these neurotransmitters.

The hypothetical role and the mechanism underlying the action of cAMP in the physiology of synaptic transmission at the synapse may be represented by the following flow sheet.
Fig. E Generalised relationship between neurotransmitters and pre- and postsynaptic receptor. $\alpha_1 =$ postsynaptic alpha adrenergic receptor, $\alpha_2 =$ presynaptic alpha adrenergic receptor, DA = pre and postsynaptic dopaminergic receptor, N = pre- and postsynaptic acetylcholine (nicotinic receptor) M = pre- or postsynaptic acetylcholine (Muscarinic) receptors (Langer, 1973).
Neurotransmitter release from presynaptic nerve endings → neurotransmitter receptor interaction → activation of adenylate cyclase in the postsynaptic membrane → accumulation of cAMP in the postsynaptic neurons → cAMP activation of a protein kinase leading to the phosphorylation of the protein constituents of the plasma membrane → phosphorylation of the plasma membrane of the postsynaptic neuron causing an alteration in the permeability properties of the membrane, resulting in a change in membrane potential of the cell → removal of the phosphate from the membrane protein by a protein phosphatase present in the synaptic membrane and the membrane potential returning to its initial value.

This scheme provides a mechanism by which cAMP, acting as the mediator for the action of some neurotransmitters may regulate the membrane properties of certain types of neurons (Greengard and Kebabian, 1974) and thereby modulate their excitability (Fig. F).

6. Neurotransmitter Removal

Several mechanisms to facilitate loss of activity for neurotransmitter substances are known to exist:

[A] Recapture of the released transmitter through neuronal uptake,

[B] Extraneuronal uptake of transmitter,
Fig. F Model of the proposed mechanism by which cyclic AMP acts as the mediator of dopaminergic transmission (Greengard and Kebabian, 1974).
[C] Metabolism of the neurotransmitter,

[D] Diffusion away from the receptor sites and/or binding to silent receptors and subsequent metabolism.

Route [A], according to Iversen (1974), is the most economical and selective uptake mechanism which exists for the neurotransmitter amines. The neuronal uptake mechanisms for \(^3\)H-NE uptake has been demonstrated and studied in synaptosomal particles (Coyle and Snyder, 1969; Bogdanski and Brodie, 1969). Iversen (1974) termed this uptake process as uptake\(_1\), which appears to have identical properties in the NE containing neurons in the CNS. The uptake\(_1\) process for NE has the following properties:

1. It involves a sodium-dependent carrier system dependent on metabolic energy and on the continued functioning of membrane Na\(^+\)K\(^+\)ATPase.

2. Uptake\(_1\) is saturable and has a very high affinity for NE.

3. In rat the uptake\(_1\) is stereochemically selective for L-NE.

4. In mammalian neurons, many other phenolic derivatives of \(\beta\)-phenylethylamine can act as substrates for uptake\(_1\). These include, norpinephrine, dopamine, tyramine and octopamine.
5. The structural requirements for uptake₁ substrates are: (a) absence of bulky N-substituted groups; (b) absence of methoxy group on phenolic substituents; (c) presence of at least one phenolic hydroxyl group.

6. Affinity for uptake₁ is decreased by the presence of bulky N-substituents by methoxylation of phenolic hydroxyls and by the presence of a β-hydroxyl group on the side chain.

7. Affinity for uptake₁ site is increased by the presence of phenolic hydroxyl group, especially in the para and meta position, as also by α-methylation of the α-carbon of the side chain.

Similarly, the dopamine containing neurons in the mammalian CNS possess their own specialised DA uptake system, having the following properties:

1. It is temperature sensitive (Holz and Coyle, 1974).

2. In synaptosomes, the uptake is linear upto 2 min (Holz and Coyle, 1974) and is proportional to the amount of synaptosomal protein (Horn, 1976).

3. The uptake is dependent on the concentration of Na⁺ and to a certain extent on that of K⁺ (Holz and Coyle, 1974), and hence dependent on metabolic energy and the continued functioning of membrane Na⁺K⁺ATPase.
4. The DA uptake system displays Michaelis-Menten kinetics and has a $K_m$ of $1.3 \times 10^{-1} \ M$ for DA and a $V_{max}$ of $25.3 \ p\ moles/100 \ \mu g\ protein/2\ min$ at normal physiological $Na^+$ and $K^+$ (Holz and Coyle, 1974) concentrations.

5. Various $\beta$-phenylethylamine analogues are taken up by dopaminergic neurons (Ross, 1976).

6. DA uptake system is less sensitive than NE uptake system to inhibition by tricyclic antidepressant (Horn et al., 1971).

7. In general, the structure-activity relationship for $\beta$-phenylethylamine derivatives such as $3^H$-DA uptake are similar to those described for NA uptake (Horn, 1976).

(a) Phenolic hydroxyl group in the para and/or meta position enhances the uptake site affinity,
(b) $\alpha$-methylation increases affinity, (c) $\beta$-hydroxylation decreases affinity, (d) Mono- or di-$N$-methylation decreases affinity, (e) O-methylation of phenolic hydroxyls produce a marked decrease in affinity.

Route [B], the extraneuronal uptake or the uptake$_2$ (Iversen, 1965) of catecholamine is by a different trans-
port system in extraneuronal peripheral tissues such as glial cells, vascular smooth muscle, cardiac muscle and certain glandular tissues. The characteristics of the uptake\textsubscript{2} system are as follows:

1. Uptake\textsubscript{2} occurs at higher concentration of the amine in the perfusion fluid (concentration $> 1 \mu g/ml$).

2. Uptake\textsubscript{2} has much lower affinity for NE ($K_m = 290 \mu M$).

3. Uptake\textsubscript{2} is less Na-dependent.

4. Uptake\textsubscript{2} shows no stereochemical selectivity for (-) or (+) NE.

Although the extraneuronal sites have low affinity for catecholamines, they are far more numerous in most peripheral organs than the neuronal uptake sites and, thus, the actual rate of removal of catecholamines from the extracellular fluid may be greater by uptake\textsubscript{2} than by uptake\textsubscript{1}. It is anticipated that once the neurotransmitter amines are actively taken up, they are either 'uptaken' into the neuronal vesicles, or are oxidatively deaminated by monoamine oxidase.
Lai et al. (1980) reported that MAO inhibitors, clorgyline and deprenyl, inhibit the uptake of neurotransmitter amines in the synaptosomes. This finding suggests that the monoamine oxidase is also responsible, and is involved in the amine uptake mechanism.

Desaiyah and Ho (1976) observed that the catecholamine affects the Na⁺K⁺ATPase in a biphasic manner, i.e. activation at low and inhibition at high amine concentrations. Experiments in the present dissertation have also shown that amines have no direct effect on the Na⁺K⁺ATPase activity in the MAO inhibited preparations. This observation suggests that MAO is in some way involved in the regulation of Na⁺K⁺ATPase activity and the consequent uptake of catecholamines. Further experiments showed that it was the oxidatively deaminated product of MAO catalysed reaction which was responsible for the regulation of Na⁺K⁺ATPase activity.

Thus, it is clear that there may be a close correlation between the MAO/monoamines/Na⁺K⁺ATPase system in brain.

Latter part of the literature survey will highlight the discussion of the MAO/monoamine system, monoamine/Na⁺K⁺ATPase system and its regulation under various hormonal and drug induced conditions.
Monoamine oxidase (monoamine, $O_2$ oxidoreductase deaminating, EC 1.4.3.4) (MAO) is a mitochondrial enzyme responsible for the oxidative deamination of a variety of biogenic amines (Youdim, 1975). MAO in brain is of physiological importance as the enzyme inactivates transmitter monoamines, namely, dopamine, noradrenaline and serotonin. Further, any change in the enzyme activity alters the neurotransmitter function. The role of MAO has been implicated in several processes, such as affective disorders (Jain and Jain, 1973), aggressive behaviour (Consolo and Valzelli, 1970), and regulatory effect of adrenocortical studies (Callingham and Laverty, 1973).

The enzyme has been assumed to exist in multiple electrophoretic forms (Youdim et al., 1969). Because of the central role of MAO in biogenic amine metabolism and the therapeutic benefits of the inhibitors of this enzyme in clinical psychiatry, a careful analysis of the isozyme concept of MAO is pertinent (Jain, 1977). Therefore, the focus in the 'introduction' would be on the interpretation of the findings, rather than on the exhaustive survey of the literature.
1. **Substrate Selective Monoamine Oxidase**

Johnston (1968) has suggested the existence of multiple forms of MAO, based on the studies with its irreversible inhibitor, clorgyline. It has been proposed that MAO can be classified into two types A and B according to their inhibitor sensitivity and substrate specificity (Murphy, 1978). Type A MAO is found to be solely responsible for the deamination of 5-HT and shows high sensitivity to clorgyline, while type B MAO metabolises 2-phenylethylamine (PEA) and benzylamine (BA) and is less sensitive to clorgyline. Knoll and Magyar (1972) have shown that type B MAO is highly sensitive to another irreversible inhibitor, deprenyl. However, recent reports have questioned the concept of 'multiple form' of MAO, and strong evidences have come forth favouring a 'one molecular entity' of the enzyme (Houslay et al., 1976; Jain, 1977; Ekstedt, 1979; Suzuki et al., 1979; Houslay, 1980; Schurr, 1982).

2. **Electrophoretic Patterns and Phospholipid Connection**

The multiple form or isozyme concept of MAO received impetus, through the demonstration of several enzyme bands when solubilised MAO was subjected to
polyacrylamide gel electrophoresis (PAGE). But the electrophoretic mobility was questioned, owing to several methodological and technical considerations.

Costa and Breakefield (1979, 1980) and Nagy and Salach (1981) have demonstrated that the electrophoretically separable MAO differ in their phospholipid content, which may give rise to multiple Rf values on polyacrylamide gel electrophoresis. Supportive evidence has come from the studies of Edward and Pak (1979) who separated two distinct sites for the binding of MAO inhibitor pargyline, probably on two distinct subunits of equal or almost equal molecular weights.

The role of phospholipids in the determination of the distinct MAO activities has attracted much attention since 1973. To quote Tipton et al. (1973), 'Since lipid binding can cause significant alterations in the properties of the enzyme (MAO), it is tempting to conclude that the existence of the enzyme in different environments could give rise to portions of the enzyme with different properties in a single tissue.'

Huang (1980) and Huang and Faulkner (1980, 1981) have suggested that, in an intact mitochondrial pre-
parations from rat brain, the functional states of the multiple forms of MAO are regulated by distinctly different lipid protein interactions in situ. They concluded that (i) a temperature-induced conformational change in MAO-A was due to an additional lipid-protein interaction originating from the tightly bound lipid shell or an intrinsic alteration in the enzyme protein, (ii) that for MAO-A enzyme, the active site was buried in the hydrocarbon core and the functional state was intimately modulated by the fluidity of the hydrophobic region proximal to the polar surface. For MAO-B enzyme, the active site was situated closer to or partly in the peripheral hydrophobic region and its functional state was strongly dependent upon the ionic and polar characteristic of the surface layer of the membrane, (iii) that phosphatidylinositol uniquely stimulated the MAO-A activity to 80 per cent over that in the original intact mitochondria.

Only a single species of the enzyme was found by Jain and Sands (1974) using human brain tissue, when the enzyme was solubilised by a non-sonication procedure. Even in the present studies, no evidence for non-homogeneity of MAO, either on DEAE cellulose column or on polyacrylamide gel, was obtained when the MAO was solubilised using only the sonication method.
Similarly, Norstrand and Glantz (1973) reported only a single moiety of the solubilised human-liver MAO by ultracentrifugation as well as by gel electrophoresis. Nagatsu et al. (1969) found only a single, almost symmetrical, peak on Sepharose 4B column using solubilised human brain preparations.

Even the differential effect of clorgyline and deprenyl on MAO activity was envisaged to emerge from the differences in partition coefficient of the two forms, and their abilities to affect the fluidity of the phospholipids in the microenvironment of MAO (Houslay, 1977).

The most convincing evidence for the support of the 'one-molecular entity' concept comes from the study of Houslay (1980). This study shows that the phospholipid substitution of the mitochondrial MAO can lead to the abolition of clorgyline selective inhibition without alterations in ratio of the A/B forms of the enzyme.

3. Kinetic and Mechanistic Studies

There are a number of kinetic studies which suggest MAO to be a single enzyme species with multiple active centres or binding sites (Huszti, 1972; Severina, 1973; Houslay et al., 1974; White and Wu, 1975).
The rationale is based on the Dixon and Webb's interpretation, as described in detail by Jain (1977). Using electron spin resonance technique for the estimation of MAO activity, Huang et al. (1976) observed a binary curve, which does not necessarily imply a multiple form.

Studies pertaining to the mechanism of action of several MAO inhibitors, viz., clorgyline and deprenyl, show that these inhibitors react in a stoichiometric fashion with the FAD prosthetic group of MAO, 1 mol of inhibitor binding to 1 mol of flavin (Jain, 1977). This is in contradiction to the selectivity and specificity of these inhibitors. For, if clorgyline were specific inhibitor of MAO A and deprenyl of B, it would hardly seem consistent that these inhibitor combine with MAO in a strikingly similar fashion.

Jain (1977) is of the view that in order to maintain multiple form postulate based on differential action of these inhibitors, some molecular basis other than the similarity in binding of these inhibitors to flavin moiety of MAO would have to be established. This is particularly difficult, since it is beyond doubt that MAO is inactivated via its flavin cofactor
(Edmondson and Singer, 1976). There are no evidences, however, that pargyline, clorgyline or deprenyl interact with sulphydryl group.

Recently, a considerable amount of monoamine oxidase activity was reported to be present in the soluble fraction of tissue homogenates (Mayanil and Baquier, 1982; Copeland et al., 1983). Data from the present dissertation show that the MAO S or the cytosolic MAO has some properties different from the mitochondrial enzyme. Further molecular mechanistic studies and immunological characterisation of this cytosolic MAO becomes extremely pertinent for synthesis and development of new antidepressant drugs with greater specificity, potency and efficacy.

VI. Na\(^+\) AND K\(^+\)-DEPENDENT ADENOSINE TRIPHOSPHATASE

Na\(^+\)K\(^+\)ATPase (Na\(^+\), K\(^+\) ATPase, Mg\(^{2+}\) dependent ATP phosphohydrolase, EC 3.6.1.3) catalyses the hydrolysis of ATP to form ADP and inorganic phosphate.

\[
\text{ATP} \xrightarrow{\text{ATPase}} \frac{\text{ADP} + \text{P}_i}{\text{Mg}^{++}, \text{Na}^+, \text{K}^+}
\]

1. Mechanism of the Enzymatically Catalysed Hydrolysis of ATP

Post et al. (1969) proposed a simple stepwise mechanism of the enzymatically catalysed hydrolysis of ATP.
Step 1: Binding of ATP to the Enzyme

The 6-amino group in the purine ring, the 2-OH group in ribose, and the $\beta$-$\gamma$-pyrophosphate group have been indicated as binding groups on the ATP molecule (Hegyvary and Post, 1971; Jensen and Norby, 1971).

Binding sites on the enzyme appear to be an arginine guanidino group (De Pont et al., 1977) probably binding the $\beta$-$\gamma$-pyrophosphate group of ATP, a tyrosyl-OH group (Cantley et al., 1978; Martonosi and Feretos, 1964) and a cysteinyl-SH group probably interacting with the purine group of ATP (Shamoo and Maclennam, 1975).

Step 2: Phosphorylation

$\text{Na}^+$ and $\text{Mg}^{++}$ ion dependent phosphorylation of the enzyme is then accompanied by breakdown of ATP to ADP resulting into a phosphorylated intermediate $E_1\sim P$. 

\[
\begin{align*}
\text{E} + \text{ATP} & \rightarrow \text{E-ATP} & \text{[1]} \\
\text{E-ATP} \xrightarrow{\text{Na}^+, \text{Mg}^{++}} \text{ADP} + E_1\sim P & \text{[2]} \\
E_1\sim P \xrightarrow{\text{Mg}^{++}} E_2P & \text{[3]} \\
E_2\sim P \xrightarrow{\text{K}^+} E + P_i & \text{[4]}
\end{align*}
\]
Step 3: Transformation of the Enzyme Conformation

There is a conformational change of the enzyme molecule from $E_1 \Leftrightarrow P$ to a state of 'low energy' $E_2 \Leftrightarrow P$. It does not react with ADP, but gets decomposed in the presence of $K^+$. Although the existence of $E_1 \Leftrightarrow P$ to $E_2 \Leftrightarrow P$ intermediates have been questioned (Klodos and Skou, 1977), recent tryptic digestion studies (Jorgensen, 1982) and intrinsic protein fluorescence studies (Chetverin et al., 1980) have revealed the cation induced transition of the enzyme conformations.

Step 4: $K^+$ Stimulated Dephosphorylation

During this process, the $K^+$ binding sites turn inward and the enzyme returns to its original conformation (Siegel and Albers, 1967).

2. Effect of Inhibitors

Digitalis glycosides, sulphydryl reagents, oligomycin, butanedione, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole and vandate are known Na$^+K^+$ATPase inhibitors (Schwartz et al. 1975). The present dissertation will deal only with ouabain, which represents the class of digitalis glycosides.
Ouabain does not inhibit Ca-Mg-ATPase (Schatzmann, 1975), anion-sensitive ATPase (Sachs et al., 1965) and K-H-ATPase (Ganser and Forte, 1973). It inhibits binding of ATP (Hansen et al., 1971) and dephosphorylation of the phospho intermediate (Sen et al., 1969) by reacting at the outer membrane side (Cladwell and Keynes, 1959). According to Skou et al. (1971), Mg$^{++}$ is essential for the ouabain-enzyme interaction. Phosphorylation by ATP or P$_i$ increases the binding rate, yielding complexes with the same dissociation constant (Schonfeld et al., 1972; Erdmann and Schoner, 1973).

The activating cations, namely, Na$^+$, K$^+$ also affect ouabain binding. Internal Na$^+$ and K$^+$, as well as external K$^+$, inhibit the ouabain binding, whereas external Na$^+$ promotes binding by competition with external K$^+$ (Hoffman, 1973). Mg$^{++}$ is required for the binding of ouabain, and that its binding affinity may be controlled by the alkali cations and vice versa (Robinson, 1972, 1974, 1976). These alkali-cation effects are related to the formation rate of the ouabain-enzyme complex. Once the complex has been formed, the alkali metal ions have little effect on the dissociation rates, provided Mg$^{++}$ is sufficiently present to impart stability to the complex (Erdmann and Schoner, 1973; Lane et al., 1973).
3. Phospholipid Involvement

Like most membrane bound enzymes, Na\(^+\)K\(^+\)ATPase also require phospholipids for its activity. Lipid removal from crude enzyme preparations by detergents, (Kimelberg and Papahadjopoulos, 1972) organic solvents (Jarnefelt, 1972) or phospholipases treatment (Goldman and Albers, 1973; Taniguchi and Iida, 1973) leads to partial or complete inactivation of the enzyme.

The effect of treatment with phospholipase A on the binding of ouabain to Na\(^+\)K\(^+\)ATPase as well as on Na\(^+\)K\(^+\)ATPase activity was studied by Taniguchi and Iida (1973) in the presence of various concentrations of ATP and other ligands. They observed that there are at least two kinds of ouabain binding conformations, one induced by \([\text{Mg}^{++} + \text{K}^+ + \text{ATP}], [\text{Mg}^{++} + \text{P}_i]\) or \([\text{Mg}^{++}]\), in which phospholipids play a role and another found in the presence of \([\text{Mg}^{++} + \text{ATP}]\) or \([\text{Mg}^{++} + \text{Na}^+ + \text{ATP}]\) in which phospholipids do not play a role.

Wheeler and Whittan (1970) suggested that the transport system would consist of a complex of Na\(^+\)K\(^+\)ATPase, protein, and phosphatidylserine. The involvement of phospholipids in active transport of ions and metabolites has been reported (Sun, 1974). It is possible
that the active uptake of catecholamine depends upon
the fine arrangements of phospholipids present at
the active sites of the synaptosomal membrane.
Hilden and Hokin (1976) observed that after full re-
placement of endogenous phospholipids of the purified
Na\(^+\)K\(^+\)ATPase by phosphatidyl choline, incorporation of
the substituted enzyme in phosphotidylcholine vesicles
leads to sodium transport.

4. Transport Functions

On energetic grounds, a circulatory carrier
mechanism seems unlikely, because it involves move­
ments of hydrophilic surfaces of protein through the
hydrophobic interior of the membrane. Alternatively,
if the mechanism invokes opening and closing of a
channel through the membrane, it must also establish
vectorially directed driving forces for diffusion of
the transported ions through the channel, and incor­
porate some device to prevent their backward diffusion.
Such a mechanism would then involve energy expenditure
in the form of ATP hydrolysis coupled to ion transport
(Wiggins, 1982a).

In an ordered construction, where proteins behave
as molecular machines, energy can be localised on
specific degrees of freedom which exchange extremely
slowly with the thermal ones. It is only then can energy, which is liberated by an energy donating reaction, ensure an energy-accepting reaction (i.e. the ion translocation) before total dissipation.

Thus, the transport and catalytic functions of the Na⁺K⁺ATPase are described in terms of a series of reversible chemical reactions which take place at specific sites on the surface of the enzyme moiety. This is followed by a change in the equilibrium conformation of the enzyme which is determined by the number and nature of groups and ions attached to its surface, and by ionic strength, medium composition, pH and temperature (Skou, 1982).

There is a rapid local equilibration with thermal degree of freedom, following a chemical reaction at a specific site on the enzyme surface. The rest of the protein is rendered 'out of equilibrium' with its active site, and then slowly relaxes to a new unique equilibrium conformation (which is thermodynamically stable) until the next chemical reaction renders it out of equilibrium and another relaxation takes place. It should be noted that each combination of chemical reaction and relaxation is a single unitary process during which the energy derived from the energy-donating
chemical reaction is stored in the protein conformational change, unable to exchange with the thermal degree of freedom, but available to perform the work of ion transport (Wiggins, 1982a).

The configurational changes of the two phosphorylated intermediates are assumed to involve generation of highly hydrogen bonded interfacial water in which all preformed ionic equilibria are displaced in the direction of less highly hydrated species, so that, depending upon the localization of that place, the Na⁺K⁺ATPase transports Na⁺ out or K⁺ in across the membrane against a macroscopic electrochemical potential gradient.

The reaction sequences described in Fig. G, involving relaxation and conformational changes are as follows:

\[3 \text{Na}^+ + E \rightarrow \text{Na}_3 E \rightarrow \text{Na}_3 E^a\] \[1\]

\[2 \text{Mg}^{++} + \text{Na}_3 E^a \rightarrow [\text{Mg} \times \text{H}_2\text{O}]_2 \text{Na}_3 E^a\]

\[\rightarrow [\text{Mg} \times \text{H}_2\text{O}]_2 \text{Na}_3 E^b\] \[2\]

\[\text{ATP} + [\text{Mg} \times \text{H}_2\text{O}]_2 \text{Na}_3 E^b \rightarrow [\text{Mg} \times \text{H}_2\text{O}]_2 \text{Na}_3 E^b \text{ATP}\]

\[\rightarrow [\text{Mg} \times \text{H}_2\text{O}]_2 \text{Na}_3 E^c \text{ATP} \] \[3\]

\[[\text{Mg} \times \text{H}_2\text{O}]_2 \text{Na}_3 E^c \text{ATP} \rightarrow \text{ADP} + [\text{Mg} \times \text{H}_2\text{O}]_2 \text{Na}_3 E^c P\]

\[\rightarrow 2 \text{Mg}^{++} + \text{Na}_3 E_1 P \ldots \] \[4\]
2 Mg$^{++}$ + Na$_3$ E$_1$ P $\rightarrow$ 3 Na$^+$ + Mg$_2$ E$_1$ P (Cyto)  
3 Na$^+$ + Mg$_2$ E$_2$ P  \[5\]

Mg$_2$ E$_2$ P + H$_2$O $\rightarrow$ P$_1$ + Mg$_2$ E$_2$ $\rightarrow$ Mg$_2$ E$^d$ \[6\]
Mg$_2$ E$^d$ $\rightarrow$ 2 Mg$^{++}$ + E$^d$ $\rightarrow$ E \[7\]

[The subscripts a, b, c, d are different conformations of the enzyme.]

Step 1, 2 and 3:

The dimeric enzyme binds Na$^+$, hydrated Mg$^{++}$, ATP in the relative positions.

Step 4:

Phosphorylation occurs to produce an out of equilibrium phosphoenzyme, which relaxes to an equilibrium conformation assumed to have the properties of the experimentally observed E$_1$ P (Wiggins, 1982a). During the relaxation to E$_1$ P, water in cleft becomes more organised and a poor solvent for highly hydrated cations. The phase change starts at the phosphorylation site and moves up the cleft between the monomers. Hydrated Mg$^{++}$ ions are more stable in normal aqueous phase than in the highly organised and hydrogen bonded phase. They, therefore, dissociate and move up the cleft, in advance of the front of ordered water.
Fig. G  The principal equilibrium conformations of the Na ATPase transporting cycle of the Na\(^+\)K\(^+\)ATPase, (a) Free enzyme, (b) the Enzyme E\(_1\) with Na\(^+\), hydrated Mg\(^{++}\) and ATP bound (c) the first phosphorylated intermediate, E\(_1\) P, the equilibrium form of the phosphoenzyme when Na\(^+\) occupies the site at the apex; ordered water surrounds both phosphorylation and cation binding sites and Mg\(^{++}\) has been concentrated at the apex (d) the actively transporting conformation, E\(_2\) P, the equilibrium form of the phosphoenzyme when Mg\(^{++}\) occupies the binding sites of apex: three Na\(^+\) per polypeptide chain per ATP hydrolysed, diffuse out through the permselective channel and the P is in contact with water of normal reactivity (Wiggins, 1982a).
Step 5:

This is the displacement of Na\(^+\) ions from their high affinity sites by Mg\(^{++}\), followed by the formation of second phosphoenzyme E\(_2\) P. Reaction \([5]\) goes from left to right because the free energy available for one Mg\(^{++}\) ion to displace 1.5 Na\(^+\) ions is 75 K Joules mol\(^{-1}\) (Wiggins, 1982b), which allows for a considerable difference in affinity. The conformation E\(_2\) P represents the equilibrium form of the enzyme in which Mg\(^{++}\) occupies the binding sites at the apex of the cleft. The ordered interfacial phase surrounds the bound Mg\(^{++}\) ions and the Na\(^+\) selective channel opens between the monomers. During the relaxation to E\(_2\) P which starts at the apex, the released Na\(^+\) ions diffuse out through the channel to the outer side of the membrane. This is a spontaneous movement because the local concentration and the local activity coefficient are both extremely high. The life time of E\(_2\) P is terminated by hydrolysis, because the aspartyl phosphate is now in contact with water of normal reactivity.

Step 6, 7:

Finally Mg\(^{++}\) ions dissociate from the sites for which they have low intrinsic affinity and the cycle continues.
The above discussed mode of Na\textsuperscript{+}K\textsuperscript{+}ATPase is termed as the Na\textsuperscript{+}ATPase mode. This mode is believed to operate in the absence of external Na\textsuperscript{+} and K\textsuperscript{+}, and therefore is not important physiologically. To impart physiological importance to the Na\textsuperscript{+}K\textsuperscript{+}ATPase mode of functioning, a rapid charge compensation through a rapid fluxes of K\textsuperscript{+} inward is done by the K\textsuperscript{+} ATPase mode of Na\textsuperscript{+}K\textsuperscript{+}ATPase.

The reactions and relaxations of the K\textsuperscript{+} compensating mode are therefore as follows:

\begin{align*}
3 \text{K}^{+} + \text{E} & \rightarrow \text{K}_{3} \text{E} \rightarrow \text{K}_{3} \text{E}_{2} \\
2 \text{Mg}^{++} + \text{K}_{3} \text{E}_{2} & \rightarrow \text{Mg}_{2} \text{K}_{3} \text{E}_{2} \rightarrow \text{Mg}_{2} \text{K}_{3} \text{E}_{2}^{a} \\
\text{P}_{1} + \text{Mg}_{2} \text{K}_{3} \text{E}_{2}^{a} & \rightarrow \text{Mg}_{2} \text{K}_{3} \text{E}_{2}^{a} \text{P} \rightarrow 3 \text{K}_{1} + \text{Mg}_{2} \text{E}_{2}^{b} \text{P} \\
\text{H}_{2}\text{O} + \text{Mg}_{2} \text{E}_{2}^{b} \text{P} & \rightarrow \text{P}_{1} + \text{Mg}_{2} \text{E}_{2}^{b} \rightarrow \text{Mg}_{2} \text{E}^{b} \\
\text{Mg}_{2} \text{E}^{b} & \rightarrow 2 \text{Mg}^{++} + \text{E}^{b} \rightarrow \text{E}
\end{align*}

(The subscripts a,b represent a conformational state of the enzyme.)

The hydrated bound Mg\textsuperscript{++} ions have been omitted in this case, because they remain associated throughout the cycle (Wiggins, 1982b).
Fig. H The $K^+$ compensating cycle: (a) the free enzyme, $E$; (b) the enzyme $E_2$ with its external binding sites occupied; (c) $E_2$ with $Mg^{++}$ occupying both sets of inner cation sites; and (d) $E_2 P$ with open channel through which $K^+$ ions have diffused in response of the field generated by pumps units operating in the NaATPase cycle. This phosphoenzyme is rapidly hydrolysed, but slowly relaxes back to $E$. (Wiggins, 1982b).
It is of interest to note that $K^+$ and less highly hydrated cations have lower activity coefficients in the ordered aqueous phase. During the lifetime of $E_2^P$, which is regulated by the magnitude of the transmembrane potential difference, three $K^+$ ions dissociate from the binding sites and diffuse into the ordered water in the cleft in response to the electric field generated by other pump units which are transporting $Na^+$ outward. This open channel allows rapid movement of compensating charge, so that the lifetime of $E_2^P$ in the NaATPase mode and $E_2^{'P}$ in the $K^+$ compensating mode are both short and the turnover of the ATPase is rapid (Wiggins, 1982b).

Following hydrolysis of $E_2^P$, relaxation of $E_2$ to $E$ is slow (Karlish, 1978), unless it is accelerated by binding of nucleotide. Since the nucleotide binding site of the conformation $E_2$ has a low affinity for ATP, millimolar concentrations of ATP must be used. This explains the inhibition of $Na^+K^+$ATPase activity by increasing concentrations of $K^+$ at low ATP concentration. The compensating cycle is slow, and as the concentration of $K^+$ increases, more pump units engage in that cycle and the relative number of $Na^+$-pumping units decreases. The NaATPase is fully active at $\mu$M concentrations of
ATP because $E_2$ P has never had its external binding sites occupied and has none of the characteristics of $E_2$. Following hydrolysis, it rapidly relaxes to $E$.

The important features of the $Na^+K^+ATPase$ model operating in its normal $Na^+/K^+$ exchange mode are:

1. Coupling of $Na^+$ efflux to $K^+$ influx is predominantly electrical, although there is an additional component provided by the increased affinity of $K^+$ for the ordered water, resulting in a spontaneous influx of $K^+$ through the channel of $E_2$ P even in the absence of an applied field generated by the Na pumping units. This $K^+$ compensating cycle of the pump confers a rapid turnover on the Na pump.

2. The number of $K^+$ ions moving in, in exchange for $Na^+$ ions moving out, depends only on the relative number of pump units operating in the two modes, and not on the number of internal and external binding sites. For example, if for every three units pumping three $Na^+$ out per ATP hydrolysed, there are only two pump units allowing influx of 3 $K^+$ ions each. Thus the physiological ratio of $Na^+: K^+: ATP = 3: 2: 1$ is established.
VII HORMONAL REGULATION

1. **Insulin**

Much effort has been spent trying to elucidate the mechanism of insulin action, since its discovery by Banting and Best in 1921. The action of insulin has been primarily associated with the regulation of carbohydrate metabolism. Such an association is not at all unreasonable because the major therapeutic use of the hormone has been in the treatment of diabetes mellitus. That insulin also acts to regulate protein, lipid and catecholamine metabolism has also been recognised (Ashmore and Carr, 1965).

The stimulation of glucose uptake by insulin is undoubtedly the most widely investigated action of a hormone on transport. Nearly every current interpretation of glucose transport into cells is based, in parts, on results obtained from the action of insulin (Krahl, 1961; Levine, 1961). The primary action of insulin is to increase glucose uptake by increasing the intracellular transport, and this effect of insulin on cell permeability is restricted to extrahepatic tissues (Ashmore and Carr, 1965).

Following the release of insulin, which is stimulated by cAMP and Ca^{++} and mediated by cAMP-dependent protein kinase and calmodulin-dependent protein kinase
respectively (Schubart et al., 1982), the action of insulin at the cellular level according to Kahn (1979) may take place at four distinct loci: (1) the binding of the hormone to its membrane receptor, (2) transformation of this hormone-receptor interaction into some form of transmembrane signal, (3) generation of an intracellular message, (4) subsequent chemical modification of various enzymes and transport systems, which results in the final biological effect of insulin.

a) Insulin Receptors

Insulin receptor appears to be an integral membrane protein with carbohydrate components with an estimated radius of 68-72 Å, and molecular weight of 300,000 to 1,000,000 daltons. This large complex dissociates into a small species (30-40 Å) in the presence of insulin. These solubilised receptors may contain closely associated membrane proteins involved in the regulation of receptors or insulin action (Ginsberg et al., 1976; Maturo and Hollenberg, 1978; Kahn, 1979).

Czech and Massague (1982) proposed a model for the minimum subunit composition and stoichiometry of the physiologically relevant insulin receptor obtained by affinity labelling of this receptor in a variety of cell types and species. The receptor is a symmetrical
disulfide-linked heterotetramer composed of two α and β glycoprotein subunits in the configuration (β-S-S-α)-SS-(α-S-S-β). The disulfide, or disulfide linking the two (α-S-S-β) halves (Class I disulfides), exhibits greater sensitivity to reduction by exogenous reductants than those linking the α and β subunits (Class II disulfides).

Binding of insulin to the receptor complex appears to result in the formation or stabilization of a new receptor conformation as evidenced by an altered susceptibility of the α subunit to exogenous trypsin. The insulin receptor structure appears to be shared by a receptor for insulin like growth factors (Massague and Czech, 1982 a,b).

b) Insulin-Receptor Interaction

De Meyts et al. (1978) demonstrated the cooperative nature of insulin-receptor interaction. Although the bioactive site of the receptor has not yet been defined, it is suggested that it is a complex containing residues of both A and B chains which come together in the 3-dimensional folding of the molecule (Pullen et al., 1976). Insulin itself is the most important factor which regulates the number and affinity of the insulin receptors. Exposure of the cells, both in vivo and in vitro, to high concentrations of insulin results in a decrease in the number of receptors, a process which has been termed down regulation (Roth et al., 1975).
c) Transmembrane Signal and Intracellular Messengers

Currently, more attention is focused on two important possibilities: (a) that insulin interacts with its receptor, and the resultant hormone receptor complex then activates another membrane protein (effector) to generate a second 'intracellular messenger'. The effector system/second messenger, could be analogous to adenylate cyclase/cAMP, a transport protein/ion, or some other unknown processes (Kahn, 1979; Larner et al., 1982), (b) the receptor for insulin might be visualized as serving a transport function similar to that of the receptors for low density lipoprotein, transferrin etc. In this case, the receptor would aid the internalisation of the hormone and 'the second messenger' would be the internalised hormone or receptor, or a product of their degradation (Kahn, 1979).

The possible role of cyclic nucleotides in the action of insulin has been extensively studied (Kahn, 1979). More recently, it has been suggested that insulin action may be mediated by some form of cAMP independent phosphorylation reaction. Direct effects of insulin on phosphorylation of soluble proteins in intact cells have been found; however, no insulin sensitive protein kinase or phosphatases have so far been isolated. The effect of insulin on Ca$^{++}$ flux could lead
to changes in cAMP dependent phosphorylation etc. The data, however, conflict on the question of the direction of flux, and there is no coherent picture of the problem (Kahn, 1979).

Seals and Czech (1982) are of the view that binding of insulin to its receptor activates a membrane structure, resulting in the increased release into the cell of a small peptide fragment by proteolytic cleavage. The released peptide is proposed to modulate several cellular enzymes such as pyruvate dehydrogenase and glycogen synthase by interacting with phosphoprotein phosphatase or protein kinase or both.

Jaret et al. (1982) have proposed a schematic mechanism of insulin action through the control of phosphorylation by two mediators $M_1$ and $M_2$ that have been shown to exist by Larner's group (Chen et al., 1980). This schematic representation is as follows:
Insulin + Receptor

Insulin Receptor Complex

? Coupling Mechanism

Second Messengers or Chemical Mediators

1. Transport processes
2. Nuclear processes

\[ M_1 \leftrightarrow M_2 \]

\[ \downarrow \text{Phosphorylation} \]
\[ \uparrow \text{cAMP dependent protein kinase} \]
\[ \uparrow \text{Phosphoprotein phosphatase} \]
\[ (\text{No effect on cAMP independent kinase}) \]

\[ \uparrow \text{Phosphorylation} \]
\[ \uparrow \text{cAMP dependent kinase} \]
\[ \uparrow \text{cAMP independent kinase} \]
\[ \uparrow \text{Ca}^{++} \text{ dependent kinase} \]
\[ (\text{Calmodulin mediated}) \]
\[ \downarrow \text{Phosphoprotein phosphatase} \]

Enzymatic Changes

Metabolic effects
Goldfine et al. (1977), Gorden et al. (1978), Bergeron et al. (1977), Schlessinger et al. (1978) are of the view that after insulin binds to the cell, it undergoes some form of compartmentalisation or internalisation and that is, perhaps, very significant in the action of the hormone.

d) Insulin Regulation of MAO/Na\(^+\)K\(^+\)ATPase System in Brain

Fernstrom and Wurtman (1971) and Tagliamonte et al. (1975) have shown that insulin administration increases the concentration of tryptophan and tyrosine in brain, while decreasing that of tyrosine and free tryptophan in serum (Lipsett et al., 1973), implying thereby that lack of insulin might decrease the transport of these two amino acids from blood to brain via the blood brain barrier (Fernando et al., 1976; De Montis et al., 1977).

With this decrease in the flux of tryptophan and tyrosine amino acids into the brain from blood, the precursor pool for catecholamine biosynthesis is decreased and may result in the lowering of the activities of brain tyrosine hydroxylase and tyrosine decarboxylase (Shohmori et al., 1979), and consequently, a reduction in catecholamine synthesis and subsequent fall in brain monoamine levels. This may result in the lowering of MAO activity due to lack of substrate.
In the present series of experiments a reciprocal relationship between MAO and Na\textsuperscript{+}K\textsuperscript{+}ATPase activity has been shown in brain (Mayanil et al., 1982 a,b). Catecholamines are reported to influence Na\textsuperscript{+}K\textsuperscript{+}ATPase activity in brain in a biphasic manner, i.e. stimulation at low concentrations and inhibition at high concentrations (Desaiah and Ho, 1976). Since catecholamines are direct substrates of monoamine oxidase (Fowler et al., 1978), the role of this enzyme in the regulation of Na\textsuperscript{+}K\textsuperscript{+}ATPase has to be considered.

Moreover, inhibition of MAO A and MAO B by clorgyline and deprenyl also affects the uptake of monoamines by rat brain synaptosomal fraction (Lai et al., 1980). The data from this dissertation also shows that the catecholamine dependent Na\textsuperscript{+}K\textsuperscript{+}ATPase activity is regulated by catecholamine derived aldehydes.

2. Thyroid Hormones

Voluminous literature is available on the thyroid hormones and their role in the growth and biochemical maturation of the developing brain (Sterling and Lazarus, 1977; Tata, 1966; Balazs et al., 1968; Cocks et al., 1970). The distribution of hepatic metabolites and control of pathways of carbohydrate metabolism in liver and adipose tissues has also been shown to be affected
by thyroid hormones (Baquer et al., 1976). However, the biochemical basis of thyroid hormone action is not well understood at present. The recent recognition that thyroid hormones initiate the formation of new RNA after interaction with specific nuclear sites can serve as the operational basis for additional studies (Oppenheimer and Surks, 1975).

A large body of circumstantial evidence suggests that the basic unit of thyroid hormone action is the T₃ nuclear receptor complex (Schadlow et al., 1972). This complex stimulates the formation directly or indirectly, of a diversity of mRNA, and according to Oppenheimer (1979), there is a generalised increase in mRNA as well as a disproportionate increase in a limited number of RNA sequences. Highly selective alterations in sensitivity to T₃ nuclear receptor complex may occur at specific target genes. Metabolic factors and hormones also participate in such regulation.

In a given tissue, e.g. brain, alteration in the total number of receptor sites has not been shown to be useful as an index of thyroid hormone response, and local modulation of the response to T₃ receptor complex by a variety of factors other than T₃ may be carried out at a receptor level (Oppenheimer, 1979).
Ismaili-Beigi and Edelman (1971) showed that a large proportion of $T_3$ induced increase in oxygen consumption in tissue slices is due to a stimulation of sodium pump, implicating the role of sodium and potassium-dependent ATPase. However, the multiple action of thyroid hormones cannot be understood exclusively in terms of increased respiration, as adult brain fails to respond to $T_3$ with the characteristic increase in respiration, even though brain has intermediate number of nuclear receptors.

Schwartz and Oppenheimer (1978), in the study of the ontogenesis of brain receptors, observed a brisk increase in the concentration of $T_3$ nuclear receptors from two days prior to birth to the second day after birth. At this time, the concentration of receptor sites per mg DNA as well as the content of receptors per g of tissue was approximately equivalent to the corresponding value in adult liver. Were the failure to respond to $T_3$ with increase in oxygen consumption due solely to a diminished number of nuclear sites, one might anticipate that the neonatal brain, in contrast to the adult brain, would respond with a definite increase in oxygen consumption (Oppenheimer, 1979). $T_3$ failed to increase oxygen consumption either in the neonatal or in adult brain (Reiss et al., 1956). Thus, oxygen consumption is not an approximate parameter for measuring thyroid hormone action in this tissue.
a) Initiation of Hormone Action at the Nuclear Site

Findings that support the concept, that thyroid hormone binding sites act as true receptors involved in the initiation of thyroid hormone action, are:
(1) low capacity, high affinity characteristics (Oppenheimer et al., 1974a), (2) identity as nuclear non-histone protein (Surks et al., 1973), (3) apparent universality of distribution in thyroid hormone responsive tissues (Oppenheimer et al., 1974a,b), (4) T₃ analogues: correlation between nuclear binding and thyromimetic effects (Koerner et al., 1975), (5) the correlation between nuclear occupancy and biologic response support the relevance of the nuclear sites in the initiation of thyroid hormone action.

If T₃ were to interact with nuclear sites, in a biological meaningful fashion, one would anticipate characteristic biochemical changes in the nuclei after T₃ binding by the receptor. These changes are:
(a) increase in the rate of formation of polyadenylate (Poly A)-containing Hn-RNA and mRNA (Dillman et al., 1978),
(b) increase in RNA polymerase activity (Tata et al., 1963; Viarengo et al., 1975),
(c) increase in protein kinase activity (Kruh and Tichonicky, 1976),
(d) alteration in composition of nuclear protein (Bernal et al., 1978).
b) Thyroid Hormone Regulation of MAO/Na\textsuperscript{+}K\textsuperscript{+}ATPase System in Brain

Earlier reports have shown that T\textsubscript{3} does not affect the brain monoamine oxidase activity at any age (Hap et al., 1967). Sourkes et al. (1977) are of the opinion that MAO activity is not increased by thyroid hormones in brain of adult rats, although T\textsubscript{3} increases the formation of covalently bound flavin in the cerebra of adult rats (Pinto and Rivilin, 1979).

But contrary to the above reports, the data obtained by Engstrom et al. (1974) imply increased turnover of brain catecholamines following pretreatment with thyroxine, as well as an increased sensitivity of brain catecholamine receptors. This is substantiated by three findings.

1. Increased accumulation of DOPA in the brain following inhibition of its decarboxylation,

2. Increased accumulation of homovanillic acid in the brain following inhibition of its elimination by probenecid,

3. Increased rates of accumulation and disappearance of [\textsuperscript{3}H]DA and [\textsuperscript{3}H]NE after injection of [\textsuperscript{3}H]tyrosine.
This increase in catecholamine turnover would imply that the synthesis and the degradative pathway of catecholamine metabolism are active. A decrease in the MAO activity in brain, observed in the thyroidectomised rats (Kim et al., 1979), further substantiates the rapid turnover of catecholamines in adult rats after T3 injection.

Further support in favour of the modulation of MAO activity by thyroid hormones comes from the in vitro study of Asaad and Clarke (1978), who postulated that thyroid hormones and their metabolites either suppress an endogenous inhibitor or function to dissociate aggregated MAO.

An almost similar modulating influence on Na⁺K⁺ATPase and monoamine oxidase can be seen in hyper or hypothyroid states. Increase in catecholamine turnover by T3 implies increase in the synthesis and degradation of catecholamine and subsequent modulation of Na⁺K⁺ATPase activity by the catecholamine derived aldehydes.

VIII 6-AMINONICOTINAMIDE AND MAO/Na⁺K⁺ATPase SYSTEM IN RAT BRAIN

6-Aminonicotinamide is an antimetabolite of nicotinamide, and in the synthesis of pyrimidine nucleotide, it is incorporated into NADP or NAD, forming 6-amino-NADP
or 6-amino-NAD (Herken, 1970). These abnormally structured pyridine nucleotides are unable to act as hydrogen carriers in the dehydrogenation reactions (Dietrich et al., 1958). The 6-AN-analogue of NADP (6 AN-ADP) causes a marked inhibition of 6-phosphogluconate dehydrogenase (Herken et al., 1969) and the resultant massive accumulation of 6-phosphogluconate is thought to cause a secondary blockade of the glycolytic pathway by way of its inhibitory action on phosphogluconate isomerase (Kauffman and Johnson, 1974).

Jansson et al. (1977) have reported that 6-AN interferes with the biosynthesis of catecholamines indirectly when it blocks the pentose-phosphate pathway by decreasing the supply of reducing equivalents in the form of NADPH, which is necessary for the tetrahydropteridine cofactors of tyrosine hydroxylase. With a decrease in the supply of reducing equivalents in the form of NADPH, the catecholamine biosynthetic pathway could be rendered non-operative, and so the catecholamines would not be formed. A decrease in the MAO activity would be expected with a subsequent modulation of Na⁺K⁺ATPase activity by the catecholamine derived aldehydes.
IX MITOCHONDRIAL SIGNAL HYPOTHESIS

A mitochondrial signal hypothesis is proposed in the present dissertation, which when put to 'test' may explain the regulation of $\text{Na}^+\text{K}^+\text{ATPase}$ by neurotransmitters and the possible inclusion of monoamine/monoamine oxidase/$\text{Na}^+\text{K}^+\text{ATPase}$ system in the pathophysiology of various physiological states like diabetes and thyroid deficiency.