Discussion
DISCUSSION

NEUROTRANSMITTER CHANGES AND THEIR SIGNIFICANCE IN STREPTOZOTOCIN DIABETES

The diabetic state induced by streptozotocin (STZ) produced an elevated blood glucose level, changed the protein and cholesterol content of brain as well as other body tissues. These gross changes can have profound effects on the cellular function. The streptozotocin induced diabetic brain when analysed with HPLC showed that the brain neurotransmitter content of diabetic rats changed in various brain regions differently. The norepinephrine content increased significantly in cerebral cortex (CC) and hypothalamus (Hypo), while in brain stem (BS) and cerebellum (CB) it remained unaffected. In BS, Hypo and CS the epinephrine content increased significantly. The corpus striatum showed a decreased norepinephrine content. The noradrenergic (NA) activity was assessed by measuring the level of NE and epinephrine (EPI). The models proposed for epinephrine formation by Mefford (1987) support that increased noradrenergic activity may be observed due to increased release, reuptake, blockade or inhibition of degradation. These changes can affect epinephrine pool in the nerves. The proposed model is based on the observation that both postsynaptic and presynaptic nerves of different brain regions contain the enzyme involved in the NE→EPI conversion, phenyl-N-methyl-transferase (PNMT). The enzyme localisation and its expression are important factors deciding the formation of epinephrine in the brain. The classical concept of epinephrine neurons is now discarded and it is considered as a unique metabolite of NE with important pharmacological actions and a receptor subtype in brain which monitors and regulates its formation (Mefford
Epinephrine is recognized by the uptake system on noradrenergic terminals and so can compete for the storage in these noradrenergic neurons.

With the above modern concepts for epinephrine formation in the brain, we will try to understand this complicated NE→EPI conversion in diabetic brain regions (Table 5). The current view is consistent with the concept that the levels of 3-methoxy-4-hydroxy-phenylglycol (3-MHPG) and its sulfate ester reflect noradrenergic activity within CNS (Stewart, et al., 1994, Mefford, 1987). Previous data clearly documented that noradrenergic neurons within specific hypothalamic nuclei and other CNS regions are hyperactive in diabetes (Bitar et al., 1987). In the cerebral cortex region the increase in NE is not accompanied by an increase in EPI and also a decreased turnover rate from NE→EPI. This decreased turnover may force us to think that NE→EPI turnover ratio decreased is the reason for increased NE level. But this may not be the actual scene. If a decrease in NE→EPI conversion is the prevailing condition in diabetic CC, we could expect an absence or decrease of EPI along with this. From the present condition of no change in the EPI content it seems that in the noradrenergic nerves there is an increased uptake of NE or a reduced release of NE occurs.

Similarly in the hypothalamus, the NE content along with EPI content increased while NE→EPI turnover rate also increased. This may be interpreted as a possible high noradrenergic activity and increased NE→EPI metabolic pathway in diabetic hypothalamus.

In the brain stem the NE content remained unchanged while EPI content increased. The turnover rate showed a decrease. A possible explanation for this situation is a changed activity or expression of the enzymes involved in the conversion of NE→EPI.
The striatal noradrenergic activity of a decrease in NE and an increase in both EPI and NE→EPI turnover rate explains a higher noradrenergic activity which resulted in an increased turnover rate and thus an increased formation of EPI.

The increased noradrenergic activity in the brain ventral tegmental area has been attributed to increased locomotor activity in the rats. In a recent observation a similarity between Schizophrenic and diabetic brain state was argued (Holden and Mooney, 1994). The brain changes observed in diabetics and Schizophrenics manifest a wide range of similar disturbing physiological symptoms, such as impaired sexual function, temperature control, low blood pressure, disrupted sleep patterns, excessive thirst, poor memory, insensitivity to pain and chronic unhappiness. Hyperthermia, hyperlocomotor activity, hyperphagia associated in diabetes attributed to changed noradrenergic nerve activity in the diabetic brain (Bitar, et al., 1987).

The dopaminergic neuronal activity in diabetic brain regions showed an increase in its activity in all the brain regions examined. The most significant change of dopaminergic neurons observed is in striatum. The striatal dopamine and its metabolites in diabetic rats have been reported (Lim and Lee, 1995). This increase in the dopamine level of synaptosome is attributed to the decrease in the release of dopamine in hyperglycemic state. They also observed a decrease in the monoamine oxidase activity in striatal synaptosomes. In our study content of DA and HVA in striatal region of diabetic rats showed a significant increase and significant decrease respectively (P<0.05; -90%). The in vitro observations in striatal tissues showed a decrease in release of dopamine in 14 day old rats. The observed increase in dopamine content in our study indicate the synaptosomal
dopamine which accumulated due to an abnormality in the release. A decrease in HVA content may be due to the decreased release.

The overall increased dopaminergic activity may produce many physiological manifestations. The dopamine turnover in the limbic structures of diabetic rats were shown to increase significantly. Haloperidol- a D₁ receptor antagonist reduced locomotor activity in diabetic mice (Kamei et al., 1994). This finding bear importance since the observed dopamine increase in hypothalamic area may play a role in behavioral changes associated with diabetics. In another study the yawning behaviour was analysed in four week old STZ-induced diabetic Wistar rats (Heaton and Varrin, 1993). Dopamine changes were observed and an associated significantly lower rate of yawning was observed at 4 weeks of diabetes. The dopamine content thus may have effects on behaviour during diabetes.

In our study, the diabetic brain serotonergic activity was assessed by its ratios to both its precursor 5-Hydroxytryptophan (5-HTP) and its breakdown metabolite 5-HIAA. The turnover rate calculated for the precursor molecule to respective neurotransmitter showed an increase. It indicate an accumulation of the respective precursor. In hypothalamus 5-HTP get accumulated due to some block in the synthesis. The hypothalamic turnover for 5-HT→5-HIAA was also higher. Thus both these can result in a decreased serotonin level in hypothalamus. An altered blood brain barrier (BBB) was observed in diabetic condition which result in a decreased content of aminoacids such as tryptophan, phenylalanine, tyrosine, methionine and lysine (Mans et al., 1987). A lack of response to dietary carbohydrate or protein on brain tryptophan and serotonin in diabetic rats and an altered blood brain barrier may be an important factor bringing out the altered serotonergic function (Crandall and Fernstrom, 1980 & 1983). In similar studies on serotonin content measured in diabetic rat hypothalamus revealed a reduced rate of
serotonin synthesis within the brain (Crandall and Fernstrom, 1983). In diabetes hypothalamus, it was reported to have a decreased serotonin content (Bitar et al., 1987 & 1986). The significant finding from our study is that the 5-HT→5-HIAA degradation pathway was increased which resulted in a decreased serotonin and an increased 5-HIAA content.

Hypothalamic serotonergic activity could produce altered pituitary hormone release. It is reported that in diabetes pituitary hormone secretion is altered (Locatelli et al., 1985). Clonidine (0.15 mg/kg iv) failed to evoke a GH release in streptozotocin diabetic rats. This study suggests an impaired function of noradrenergic pathway controlling GH release in diabetic hypothalamus.

The studies carried out in other brain regions revealed that serotonergic neuronal alteration is not confined to hypothalamus alone. The CC content of 5-HT increased and 5-HIAA content remained unchanged and the turnover rate of 5-HT →5-HIAA decreased. This stand well as an explanation for the increased accumulation of 5-HT.

The brain stem results show an increase in serotonin content which is due to precursor and breakdown metabolite alteration. 5-HTP increased while 5-HIAA decreased. Turnover rates calculated for serotonin precursor and metabolite clearly showed that there is a defect in metabolic pathway of serotonin in diabetic BS region.

Cerebellum serotonin activity was not predictable due to the reason that 5-HT level was not detectable in all the samples analysed. Also that this region is more rich in GABAergic neurons (Dakshinamurti et al., 1990)
The CS serotonergic synthetic pathway seems to be active as revealed by the high content of 5-HTP and the increased turnover rate. This region is considered to be the control centre of behavioral function in animals. So changes in serotonergic activity observed in the present study may be related to behavioral changes associated with diabetes.

Thus, our study reveals that though previous studies generally conclude that in diabetic brain the rate of serotonin synthesis is reduced, but the way by which the different brain regions respond to this disturbance are different. It is known that serotonin precursor molecules and their entry across the BBB is impaired. Also, the changes in the brain level of each aminoacid paralleled the changes in the blood ratio of each aminoacid to the sum of the other aromatic and branched chain aminoacids. Crandall and Femstrom, (1983) concluded from their observations of occasional decrease of tryptophan and tyrosine in blood of diabetic rats that the changes in the brain levels of aminoacids may influence the rates at which they are consumed in brain. It may be true that tryptophan and their availability in the brain is decreased during diabetes but the serotonergic pathways in different brain regions respond differently to this altered state. It is also possible that not only the altered state of serotonin metabolism is attributable to the availability of its precursor, but also, some other nerve impairment produced by hyperglycemic state.

WHY HYPOTHALAMIC AND BRAIN STEM REGION CHANGES IN NORADRENERGIC AND SEROTONERGIC NERVES ARE IMPORTANT?

Our study on neurotransmitters and their metabolite changes during diabetes revealed that noradrenergic and serotonergic pathways of the major neuroendocrine
The post synaptic nerves as well as presynaptic nerves contain \( \alpha_2 \) adrenergic receptors. These receptors which are present on the presynaptic nerves are autoreceptors and they regulate the release of norepinephrine from the presynaptic nerves into the synaptic cleft. The epinephrine level thus is regulated uniquely by these receptors. The increased epinephrine content despite a normal level of norepinephrine in BS may be indicating an altered presynaptic \( \alpha_2 \) receptors in the BS. In hypothalamus the norepinephrine content is still high even after an increase in the epinephrine content. This indicate an altered \( \alpha_2 \) receptor function in this region. Another important possible way by which these receptors and epinephrine content is regulated is through the PNMT gene and its expression. This
enzyme can also change the α-2 receptor function. PNMT regulate the conversion of NE → EPI and this can regulate the autoreceptor expression in the nerves.

WHY NORADRENERGIC RECEPTORS ARE STUDIED ALONG WITH SEROTONIN RECEPTORS?

The availability of new tools and new emerging fields of molecular neurobiology have helped in understanding more how nerves in the discrete brain regions interact each other. In this regard the neuropharmacological drugs, new transgenic rat models and other applications of molecular techniques to neurobiology have helped in understanding the interactions of adrenergic and serotonergic neurons. The drug called 8-hydroxy di-n-propylamino tetralin, a 5-HT1a agonist in microdialysate of ventral tegmental area (VTA) revealed a dose dependent release on DA and NE (Chen and Reith, 1995). Mongeau et al., (1994) demonstrated how an ion gated 5-HT3 receptors enhances the release of [3H]NE, in preloaded slices of rat brain. Studies on pituitary-adrenocortical activation during stress showed that catecholamine and serotonin nerves together bring out this effect (Bugajaski et al. 1993, Mefford, 1987, Debreceni, 1994, Smythe et al., 1983). The evidence provided by Tian et al. (1993) clearly suggested a close association between the serotonergic and adrenergic neurons of hypothalamus. Their experiments revealed that 5-HT neurons tonically inhibit the activity of NE neurons terminating in the medial zona incerta (MIZ) and dorsomedial nucleus (DMN) of the hypothalamus, but do not influence the activity in certain hypothalamic catecholaminergic neurons. 5-HT1a receptor interactions with dopamine were also revealed by several studies (Arborelino et al., 1993).
WHY SEROTONIN AND NOREPINEPHRINE MEDIATED ADRENAL PATHWAY MAY BE IMPORTANT IN DIABETES?

Studies have shown that hypothalamic pituitary secretion in diabetes have been defective (Bestetti et al., 1995, Locatelli et al., 1985). Adrenalectomy on rats augmented NE release in the PVN neurons during stress (Mefford, 1987). Also adrenalectomy diminished the diabetogenic effect of streptozotocin in rats (Jean et al., 1994). This is attributed to a greater pancreatic insulin content. Finally it has been shown that $\alpha_2$ adrenoceptors modulate ingestive and autonomic functions in plasma glucose levels (Levin and Planas, 1993). $[^3H]p$-aminoclonidine ($[^3H]PAC$) binding to $\alpha_2$ adrenoceptors in 5 of 17 brain areas (anterior, ventromedial and arcuate nucleus) assessed by autoradiography techniques; these studies revealed a significant positive correlation and a near significant correlation in the (PVN) and lateral hypothalamus respectively. In the coming sections we will discuss the altered adrenergic and serotonergic receptor function, with emphasis on $\alpha_2$ adrenergic receptors, STZ-diabetes and their physiological significance.
NEUROTRANSMITTER RECEPTORS AND THEIR REGULATION
DURING DIABETES

In the previous part we discussed about neurotransmitter content changes in diabetes. The discussion revealed that noradrenergic and serotonergic neuronal function is altered during diabetes. A wide variety of radioligands, both agonists and antagonists, have been used to label α-adrenergic receptors in various tissues (Rapaske et al., 1987, Hoffman et al., 1979 and 1980, Tsai and Leftkowitz, 1979). Hoffman et al have shown in their studies that radioligand properties should be taken into care while choosing the radioligand for binding studies. In the present study we used (-) [3H]NE which can identify more of α-1 adrenergic receptors than any other receptors (Geynet et al., 1981). The Scatchard analysis showed that noradrenergic receptors decreased in hypothalamus. The displacement analysis using [3H]NE showed that [3H]NE is better displaced by prazosin than yohimbine. This shows that [3H]NE identifiable sites in hypothalamus contain more α-1 sites. Previous studies in genetically diabetic mice showed an increased α-1 binding sites in diabetic brain (Garris, 1990, 1995). The saturation binding curves done showed that the α-2 adrenergic receptors is representing less than 15% of [3H]NE identified receptors over the same concentration. These results show that the observed decrease in [3H]NE identifiable sites in diabetic hypothalamus could not directly indicate any appreciable change in the number of α-2 adrenergic binding sites. Also, the displacement analysis show that α-1 antagonist prazosin is a better displacing ligand than α-2 antagonist yohimbine in control hypothalamus. So the observed shift or increased potency for prazosin in displacing [3H]NE from diabetic hypothalamus can be inferred as an increase in α-1 receptors. [3H]NE Scatchard showed a decrease in adrenergic receptors. The observed decrease may be due to all other adrenergic receptors except α-1 adrenergic receptors.
The neurotransmitter epinephrine that is closely related to α-2 adrenergic receptors showed increased content in hypothalamus. But the α-2 adrenergic receptors in this brain region of diabetic rats were not down regulated; but their affinity showed an increase. As we understood earlier the synaptic regulation of noradrenergic release is inhibited or controlled by autoreceptors of α-2 type at the presynaptic membranes or on the cell body. These results may be interpreted as an abnormal regulation of α-2 receptors on this neurotransmitter.

The altered receptor affinity regulation by guanine nucleotides observed in our study emphasize an increased affinity state for the α-2 receptors observed in Scatchard analysis. The guanine nucleotide induced affinity change in diabetic hypothalamus is not demonstrable in the present study. The increased affinity observed in the scatchard analysis value showed that all receptors of α-2 type may be present in α-2 high affinity (α-2H) state. The guanine nucleotides can abolish this state of receptor and bring all the receptors to α-2 low affinity state. The observed absence of this shift may be due to an altered Gi function in the brain hypothalamus. There are many similar reports about an altered Gi protein function (Gando, et al., 1995, Mathew et al., 1994, Shindo et al., 1993, Gawler et al., 1987, Domino et al., 1992). It is Gi protein that give the capacity for receptor to remain in the high affinity state. GTP analogue which is a nonhydrolysable form of GTP, permanently disrupts the G protein from the receptor resulting in a decreased affinity state for the receptors. The detergent solubilisation of the α-2 adrenergic receptors is reported to alter the properties of receptor-agonist interactions.

Taken together all our findings on α-2 adrenergic receptors and their agonist in hypothalamus reveal a possible alteration in the Gi function. This is evidenced by an absence of GTP analogue induced steepening of the curve. This shows an impaired G protein function may keep the α-2 receptors of hypothalamus
in an increased affinity state. As we have discussed in Chapter 1 these receptors can regulate the level of epinephrine formation. So we conclude that an observed increased epinephrine content in diabetic hypothalamus may be due to an altered Gi protein induced receptor affinity state leading to a disrupted epinephrine regulation.

Sodium ions and their ability to reduce the receptor affinity was demonstrated in the membrane preparations from diabetic rats. The sodium ions alone were able to demonstrate this shift. But both Gpp[NH]p and sodium ions were not able to demonstrate the shift towards low affinity side. This again may be indicating that interaction between the α-2 receptors and G protein in diabetic hypothalamus may be altered.

Serotonergic activity assessed by serotonin content in diabetic hypothalamus is in agreement with an increased serotonin receptors. The serotonin receptors are identified using $[^{3}H]5$-HT. Many authors have referred the $[^{3}H]$serotonin as a selective $S_1$ receptor agonist in high affinity concentration (Paulose and Dakshinamurti, 1985). Our data showed a significant increase (P<0.01) in the serotonin receptors. This may be indicating an increase in $S_1$ class of receptors. The significance of this along with α-2 receptor alteration may be an important change observed in our study. The 5-HT1a agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and its effect on hyperglycemic and hypoinsulinemic effects suggested that A type of 5-HT1 recognition sites and α2 adrenoceptors mediate these changes. Our study clearly shows that hypothalamic α-2 and 5-HT1 class receptors are altered during diabetes. These changes may be important in the disease state.
How 8-OH-DPAT and serotonin receptors interact?

The 5-HT receptors has been classified based on radioligand binding studies into major four classes of receptors 5-HT¹, identifiable by tritiated 5-HT, 5-HT² by spiperone or ketanserin 5-HT³ an ion gated channel and 5-HT⁴ which is still to be pharmacologically demonstrated, but postulated to occur (Hoyer et al., 1992). It was Pedigo et al., (1981) who studied the multiple existing sites of 5-HT¹ recognition sites. The "high affinity sites" for spiperone were called 5-HT¹α, whereas low affinity sites were designated as 5-HT¹β sites. The novel centrally active 5-HT agonist 8-OH-DPAT (Hjorth et al., 1982) have a high affinity for 5-HT¹α site, which exceeds by several orders of magnitude by its affinity for either 5-HT¹β and 5-HT² recognition sites. The studies on biochemical and behavioral effects of this drug are indicative of central post synaptic 5-HT receptors and occupancy on 5-HT cell bodies. Bluet et al., (1995) showed that ACTH but not PRL control involves presynaptic interactions at 5-HT autoreceptors in raphe nuclei by 8-OH-DPAT. Chaouloff and Jeanrenaud (1987) found that α-2 adrenoceptors antagonist Idazoxan and nonspecific 5-HT antagonist, methiotepin, prevented the hyperglycemic and hypoinsulinemic effects (Chaouloff 1987). This drug helped to reveal the exact serotonin receptors and adrenergic receptors mostly involved in glucose homeostasis signals from brain hypothalamus. In our study we were able to clearly show the involvement of 5-HT¹ receptors in insulin function during diabetes.

The displacement analysis using a concentration of high affinity range of spiperone showed that spiperone is more potent in displacing the ligand in diabetic hypo than control hypo which shows an increased 5-HT¹ receptors in the high affinity range.
The neurotransmitter epinephrine content is significantly higher in the lower brain stem region. The adrenergic receptors analysed in brain stem showed a decreased affinity for the antagonist $[^3\text{H}]$ Yohimbine. Our analysis of receptor affinity may be a change in the G protein receptor association. It is not conclusive that G protein function is altered in this brain region also. The observed changes in the decreased affinity revealed by a shift to the low affinity when compared to control shows that, in diabetic state the G proteins induce $\alpha_2$ adrenergic receptors (R) that remain in a more in RL state (low affinity) state than RH (High affinity) as an adaptation to the prevailing increased neurotransmitter content. The effect of sodium ions on the receptor affinity also underline the above finding. The shift to low affinity state is more evident in diabetic state than controls with sodium ions. The abnormal added effect of both compounds, in diabetic state is unexplained. The altered receptors affinity state and the complex interaction between the compounds Gpp[NH]p and Na$^+$ ions to a heterogeneous mixture of receptor population is attributed to such effects (Michel, 1980). In general, in diabetic state the receptor affinity of $\alpha_2$ receptors affected by guanine nucleotides are intact; but more shift to the low affinity state may be an adaptation to the altered neurotransmitter activity. The observed low affinity state of $\alpha_2$ receptors in brain stem can result in a reduced $\alpha_2$ receptor mediated responses from the brain stem.

The observation of serotonergic receptors of brain stem showed that tritiated serotonin identified sites of brain stem (S1 type) showed a decreased affinity state. Generally the adrenergic and serotonergic responses from the diabetic brain stem decreased during diabetes. This may be due to down regulation brought about by the increased adrenergic and serotonergic out put observed within this region.
It has been shown that upper brain region cerebral cortex is well in communication with the lower brain regions (Paulose and Dakhinamurti, 1985). We had earlier discussed the noradrenergic and serotonergic nerve activity in cerebral cortex is increased during diabetes. Our results on the serotonergic receptors in cerebral cortex for high affinity S1 receptors showed that S1 high affinity receptors decreased in diabetic rats. The displacement data showed that in the high affinity regions ketanserin effectively displaced the ligand or it seem to be more potent displacing compound compared to controls. Ketanserin, an S2 antagonist over the same range could not appreciably displace the tritiated serotonin in control rats. This indicate a heterogeneous population of receptors in diabetic cerebral cortex. The high affinity S1 receptors estimated in Scatchard data may be indicating a decrease or switching over of serotonin receptors to a S2 receptors containing population.

Phospholipase C (PLC) is an important enzyme having a function in many receptor mediated inositol pathways. The major receptors coupled to PLC pathway include α-1 adrenergic, and 5-HT2 & 5-HT1c receptors and cholinergic receptors. The observed decrease in the PLC activity in hypothalamus may be attributed to an increased α-1 adrenergic receptors. This is supported by our displacement analysis [3H]NE binding using prazosin. But the observed change in the PLC activity may not be solely attributable to α-1 adrenergic receptors alone. The S2 class of receptors and cholinergic receptors may also be involved. The decreased total activity of PLC could be just an adaptation for the decreased receptor mediated signal transduction in diabetes or it may be possible that PLC, an enzyme with many forms, changed expression during diabetes (Casey and Gilman,1988). Our study clearly shows that phosphatidylinositol specific PLC activity is decreased in hypothalamus of diabetic rats. The first messengers of inositol phosphates and diacylglycerol pathway is PLC. The receptor coupling activate PLC and this lead to
a phosphatidylinositol break down mediated by PLC. The $\alpha_{-1}$ adrenergic receptor changes of hypothalamus can also be due to a PLC affinity changes. The other major neurotransmitter involved in this pathway is acetylcholine (Berridge and Irvine, 1984). Diabetes is known to be associated with abnormalities of membranes. The membrane lipid constitution of peripheral nerves have been shown to be altered in diabetes. In our analysis of phospholipid content of diabetic brain hypothalamus using TLC the standard phosphatidylinositol comparable spot increased on visual observation. This support our data on enzyme activity showing decrease in activity. These results clearly argue that diabetic hypothalamus has large perturbations in the PLC mediated second messenger system. Thus our study of receptors and their second messengers associated with diabetic rat brain strongly support an altered signal transduction.

The insulin treatment and its effect on receptors

In hypothalamus the insulin treatment could not reverse the altered adrenoceptor parameters. In brain stem also $\alpha_{-2}$ adrenoceptor parameter showed an increase in number compared to control (this parameter is not changed in diabetics) and reversal in the affinity is observed. These results indicate that $\alpha_{-2}$ receptor affinity changes observed in both these regions are not reversed by insulin treatment. The insulin respond in a way that maximum compensating effects are produced. The insulin treatment data indicate an abnormal recovery, i.e. sometimes it bring out the reversal but otherwise with no effect. The insulin is a good hypoglycemic agent but external insulin and its ability to reverse the metabolic and receptor changes is reported to be defective (Katovich et al., 1993). Some authors found that insulin was able to partly normalize the altered $\beta$-adrenergic responsiveness associated with diabetic state. Our study also support this. The previous study from our lab on the effect of insulin on metabolic enzymes involved
in glucose metabolism in brain tissues showed that insulin reversal on the changed parameters of enzyme activity was not complete (Preetha et al., in press.) A reversal trend is observed for the glutamate dehydrogenase activity in the brain with insulin treatment. In another set of studies an observation on ultrastructural changes in streptozotocin diabetic rats showed that insulin and other hypoglycemic agents were able to reverse the altered structural changes only partially (Das et al., 1996; Seema et al., 1996). In general, it may be concluded that although insulin is a good hypoglycemic agent, its ability to reverse the alteration at cellular level is partial. The possible reasons for this partial recovery may be either due to the insulin resistance or an alteration in the insulin receptors. Eventhough glucose levels are reversed to normal state by insulin treatment the metabolic disturbances and other brain changes are not reversed to near normal.
One of the major findings emerged from the present study was that norepinephrine conversion to epinephrine metabolic pathway is important indicator of any disturbance in the $\alpha_2$ receptor mediated pathway. Since this receptor is an inhibitory one at presynaptic sites, their regulation can affect the content of epinephrine. This is well demonstrated in our study.

The neurotransmitter changes studied over a period of time showed that their content varied over the same period. So for a comparison neurotransmitter contents of different days of analysis should take care of the day at which the animals were killed. Our study on neurotransmitter changes of serotonin content showed a pattern of decrease. The norepinephrine also showed an attenuated pattern of changes over a period of 7, 14 and 28 days. The neurotransmitter content estimated at the end of different periods can differ.

The noradrenergic activity increased and serotonergic activity decreased in the diabetic brain. The diabetic hypothalamus was reported to have altered responses in the release of pituitary hormones. Both adrenergic and serotonergic nerves are shown to be involved in this response. The studies using adrenodemedullation, stress and its effect on these two nervous system suggested that the noradrenergic neurons regulate the ACTH release positively while serotonergic neurons regulate negatively. In the diabetic state we observed changed affinity of receptors for agonists. These changes are due to an altered $G$ protein regulation of $\alpha_2L$ receptor state to $\alpha_2H$ state. The serotonin receptors of hypothalamus increased without any change in the affinity. The displacement analysis data showed that serotonin receptors of diabetic hypothalamus are more of
S1 class of receptors. The hypothalamic i.c.v. injection of 8-OH-DPAT, a 5-HT1a receptor agonist mediated hypoinsulinemia and hyperglycemia possibly through the adrenal pathway (Bitar et al., 1987). This effect was abolished by α-2 specific antagonist (Chaouloff et al., 1987). Our results show that in streptozotocin induced diabetic rats the α-2 adrenergic and 5-HT1 serotonergic class of receptors are altered. This altered receptor function can have profound effects by changing the autonomic nervous activity and releasing hormones of hypothalamic neurohormonal cells. This may control the insulin function. A defective neuroendocrine system might bring about diabetic state.

The brain stem of diabetic rats showed an altered α-2 adrenergic function. The receptor affinity was reduced in both α-2 adrenergic and serotonergic receptors. Several autonomic nerves have their regulatory centres in the brain stem. The observed receptor affinity reduction can alter the autonomic function in diabetic rats.

The other important finding of our study was that cerebral cortex of diabetic rats contain more of S2 receptors, which may lead to the altered hypothalamic function which in turn might affect the insulin function. The decrease in α-1 adrenergic receptors of diabetic hypothalamus may be related to an altered phospholipase C activity in diabetes.

The receptor studies and neurotransmitter changes in the diabetic stage caught up a momentum when it was shown in genetically obese diabetic mice noradrenergic activity is altered in the brain regions (Garris 1990, 1995). This is implicated as manifestations associated with the expression of obese gene. Also, obesity research and hyperglycemia research pointed to more involvement of genetic influence on hitherto far away processes like eating habits. The
hypothalamic lesions and its effect on hyperphagia and blood glucose regulation also have helped in understanding the role of brain in a diabetic context. The future research on this aspect should be directed more to the second messengers associated with these receptors and their physiological manifestations.

Let us conclude our discussion with a note on the hope that next century will see a place where diabetes related manifestations are better managed where neuronal control will be emphasized at the molecular level.