Summary

1. Insulin induced hypoglycaemia and streptozotocin induced diabetes in adult and old rats were used as models to study alterations in brain glutamatergic function and receptor subtype gene expression in hypoglycaemia and diabetes.

2. The body weight was analyzed to study the changes in body weight in hypoglycaemic and diabetic adult and old rats compared to control. Diabetes caused a reduction in the body weight in adult and old rats while hypoglycaemic adult and old rats did not show any significant change in the body weight.

3. Blood glucose level in the serum was measured to analyze the circulating glucose level changes due to hypoglycaemia and diabetes in adult and old rats compared to control. Diabetic adult and old rats showed increased blood glucose level. The D+IIH and C+IIH adult and old group showed significant reduction in blood glucose level. The blood glucose analysis also revealed that the D+IIH adult and old rats became hypoglycaemic at the 3rd hour and the C+IIH adult and old rats became hypoglycaemic at the 1st hour.

4. The circulating insulin level was analysed to study the changes insulin concentration in hypoglycaemic and diabetic adult and old rats compared to control. Diabetic adult and old rats showed a significant decrease in insulin level. The D+IIH and C+IIH adult and old group showed significant increase in the insulin concentration.
5. Serum T3 concentration was decreased in diabetic adult and old rats. The D+IIH and C+IIH adult and old group showed significant increase in the serum T3 content.

6. Glutamate content increased in the cerebral cortex, cerebellum, hippocampus and pancreas of adult and old diabetic, D+IIH and C+IIH rats.

7. Glutamatergic receptor functional status was analysed by Scatchard analysis using [³H]glutamate. The total glutamate receptors in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old groups increased with decreased affinity in the cerebral cortex of old C+IIH rats. Thus an enhanced total glutamate function observed in different brain regions and pancreas, had a differential effect during hypoglycaemia and diabetes.

8. NMDA receptor functional status was analysed by Scatchard analysis using [³H]MK801. The NMDA receptors in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old groups increased with no significant change the affinity.

9. NMDA mediates its action through its subunits –NMDAR1, NMDA2B, mGluR5. NMDA receptor binding parameters were confirmed by studying the mRNA status of the corresponding receptor using Real-Time PCR. NMDAR1, NMDA2B, mGluR5 receptors showed increased expression in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old rats. This shows a co-activation of NMDA receptors subunits that affect
glutamate mediated functions. This enhanced activity of NMDA receptors produce intracellular signals through activation of signaling pathways.

10. To prevent glutamate mediated excitotoxic effects it should be cleared from the extracellular space by the glutamate transporters. The gene expression of GLAST glutamate transporter was studied in hypoglycaemic and diabetic adult and old rats compared to control. GLAST glutamate transporter showed decreased expression in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old rats. This shows less reuptake of extracellular glutamate formed in the experimental condition.

11. The IP3 levels increased significantly in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old rats compared to their respective controls.

12. The cGMP levels increased significantly in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old rats compared to their respective controls.

13. The cAMP levels increased significantly in cerebral cortex, cerebellum, hippocampus and pancreas of diabetic, D+IIH and C+IIH adult and old rats compared to their respective controls.

14. Behavioural studies of the experimental groups of rats were carried out using rotarod test to assess the changes in the motor learning and motor in-coordination. The experiment demonstrated the impairment in the motor function and
coordination in the diabetic, D+IIH and C+IIH adult and old rats compared to their respective control rats by showing lower fall of time. The diabetic, D+IIH and C+IIH adult and old rats showed lower fall of time with increased rpm of the metallic rod compared to their respective control rats.

15. The increased expression of NMDAR1, NMDA2B and mGluR5 receptors in the diabetic, D+IIH and C+IIH rats obtained from the Real-time PCR was confirmed by confocal studies using receptor specific antibodies in the brain slices and pancreatic islets. Increased expression of IP3 receptors was also observed in the diabetic, D+IIH and C+IIH rats using receptor specific antibodies in the brain slices and pancreatic islets.

16. Calcium imaging results showed that dopamine at $10^{-5}$M inhibited calcium release from the pancreatic islets in hypoglycaemic condition. Dopamine D2 receptor antagonist sulpiride at $10^{-5}$ M reversed the inhibition from the pancreatic islets in hypoglycaemic condition. In the normoglycaemic condition $10^{-5}$ M dopamine increased the calcium release. Dopamine D2 receptor antagonist sulpiride at $10^{-5}$M inhibited the calcium release from the islet cells in the presence of $10^{-5}$ M dopamine. In the hyperglycaemic condition $10^{-5}$ M dopamine increased the calcium release. Dopamine D2 receptor antagonist sulpiride at $10^{-5}$ M inhibited the calcium release from the islet cells in the presence of $10^{-5}$ M dopamine.

17. Calcium imaging results showed that glutamate at $10^{-5}$M increased calcium release from the pancreatic islets in the presence of 1 mM glucose. NMDA receptor antagonist MK801 at $10^{-5}$ M inhibited the release from the pancreatic islets in the presence of 1 mM glucose. In the normoglycaemic condition $10^{-5}$ M
Summary

Glutamate increased the calcium release. NMDA receptor antagonist MK801 at $10^{-5}$ M inhibited the calcium release from the islet cells in the presence of $10^{-5}$ M glutamate and 4 mM glucose. In the hyperglycaemic condition $10^{-5}$ M glutamate increased the calcium release. NMDA receptor antagonist MK801 at $10^{-5}$ M inhibited the calcium release from the islet cells in the presence of $10^{-5}$ M glutamate and 20 mM glucose.

It is evident from our results that brain glutamate and NMDA receptor functional balance plays a major role in hypoglycaemia and diabetes management as a function age. Gene expression studies of NMDAR1, NMDA2B, mGluR5 receptor subunits and GLAST glutamate transporter showed a prominent glutamatergic functional disturbance in brain regions and pancreas of hypoglycaemic and diabetic adult and old rats. These findings have important implications for understanding the molecular mechanisms underlying memory and cognitive impairment by second messengers due to hypoglycaemia, diabetes and ageing. *In vitro* calcium release studies confirmed the regulatory role of dopamine, dopamine D2 receptor, glutamate and NMDA receptor subtypes in insulin secretion from pancreatic islets. A differential secretion of thyroid hormones in hypoglycaemia and diabetes was observed, which is an indicative of impairment in metabolic and neurological functions. The enhanced receptor activity and the second messenger cascades will lead to Ca$^{2+}$ overload and thereby excitotoxic neurodegeneration. This affected the cognitive, memory and motor ability of the experimental rats.

Thus our studies showed hypoglycaemic and hyperglycaemic effect on brain function of glutamate through NMDA receptors, second messengers and *in vitro* studies confirming the receptor subtypes functional regulation. It is suggested that the
corrective measures for the brain functional damage caused during diabetes and anti-diabetic treatment, through glutamergic receptors, have therapeutic role in the management of hypoglycaemia and hyperglycaemia.