Summary

1. Streptozotocin induced diabetic rats were used as model to study the alterations of Muscarinic M1 and M3 receptors and their regulation by insulin treatment.

2. Acetylcholine esterase (AChE) activity was used as a marker for cholinergic function. AChE activity was decreased in the cerebral cortex, brainstem and corpus striatum of old rats compared to young rats. During diabetic stage it was increased in both young and old rats in cerebral cortex, and corpus striatum while in brainstem it was decreased. In insulin treated rats the activity of the enzyme was reversed to near control.

3. Muscarinic receptor functional status was analysed by Scatchard and displacement analysis using specific [3H] ligands. Receptor analysis was confirmed by studying the mRNA status of the corresponding receptor using Real-Time PCR.

4. Muscarinic M1 receptors of old rats were down regulated in cerebral cortex while in corpus striatum and brainstem it was up regulated. Muscarinic M3 receptors of old rats showed no significant change in cerebral cortex while in corpus striatum and brainstem, muscarinic receptors were down regulated.

5. During diabetes, muscarinic M1 receptors were down regulated in cerebral cortex and brainstem of young rats while in corpus striatum they were up regulated. In old rats, M1 receptors were up regulated in cerebral cortex, corpus striatum and in brainstem they were down regulated.
Muscarinic M3 receptors were up regulated in cerebral cortex and brainstem of young rats while in corpus striatum they were down regulated. In old rats, muscarinic M1 receptors were up regulated in cerebral cortex, corpus striatum and brainstem. In insulin treated diabetic rats the activity of the receptors were reversed to near control. Muscarinic M3 receptors were up regulated in the pancreas of both young and old rats during diabetes.

6. Gene expression studies using DA D2 and β2 receptor mRNA showed an increased expressional status whereas α2A adrenergic, GABA_A, GABA_B and 5-HT_2C receptor gene expression were decreased in the cerebral cortex of young diabetic rats. Also, an increased DA D2, α2A adrenergic and 5-HT_2C expressional status was observed in old diabetic rats whereas β2 adrenergic, GABA_A and GABA_B expression were decreased in old diabetic rats.

7. Gene expression studies using glutamate receptor NMDAR1 showed decreased expressional status in the cerebral cortex, corpus striatum and hippocampus whereas it was increased in brainstem of young diabetic rats. mGlu-5 receptor mRNA showed increased expressional status in the cerebral cortex and brainstem whereas it was increased in corpus striatum and hippocampus of old diabetic rats.

8. *In vitro* studies in pancreatic islets using carbachol and muscarinic M1 and M3 antagonists showed that glucose stimulated insulin secretion were mediated through muscarinic M1 receptors in young rats whereas it was mediated through muscarinic M3 receptors in old rats at normal glucose concentration. In diabetic condition, glucose induced insulin secretion was mediated through muscarinic M1 receptors in young and old rats.
9. IP3 and cGMP content increased in young diabetic rat brain regions whereas both second messengers decreased significantly in old diabetic rat brain regions.

10. *In vitro* studies in pancreatic islets using carbachol and muscarinic M1 and M3 antagonists showed that IP3 and cGMP release were mediated through Muscarinic M1 and M3 receptors in pancreatic islets. In diabetic condition, IP3 and cGMP release were mediated through muscarinic M1 receptors in young rats whereas IP3 and cGMP release were mediated through muscarinic M3 receptors in old rats.

11. *In vitro* studies in pancreatic islets using dopamine and carbachol showed that M1 and M3 mediated IP3 and cGMP release were inhibited at lower dopamine concentration whereas IP3 and cGMP release were stimulated at higher dopamine concentration in young and old rats.

12. Serum T3 concentration was decreased in old rats compared to the young rats. During diabetes, serum T3 concentration was increased in both young and old rats. In insulin treated diabetic rats the concentration was reversed to near control.

13. NE, EPI and 5-HT content decreased in the cerebral cortex, corpus striatum, brainstem and hypothalamus of long time low dose somatotropin and insulin treated old rats compared to treated young rats. The AChE activity was increased in the cerebral cortex of long term low dose insulin and somatotropin treated young and old rats compared to saline treated young and old rats. An increased DA content was observed in corpus striatum, brainstem and hypothalamus of somatotropin and insulin treated old rats compared to saline treated old rats. No significant change in DA content was observed in somatotropin and insulin treated young rats compared to saline treated young rats.
14. Gene expression studies using muscarinic M1, M3, glutamate receptor NMDAR1, mGlu-5, DAD2, α2A, β2 adrenergic, GABA_Aα1 and GABA_B showed an increased expressional status in the cerebral cortex of long term low dose insulin and somatotropin treated young rats whereas 5-HT_2C gene expression was decreased in insulin and somatotropin treated young rats. Somatotropin and insulin treated old rats showed decreased muscarinic M1, M3, glutamate receptor NMDAR1, mGlu-5, α2A, GABA_Aα1 and GABA_B expressional status whereas DAD2 and 5-HT_2C mRNA showed an increased expressional status in the cerebral cortex when compared to saline treated old rats. Our studies on long term low dose treatment of INS and STH improved the cholinergic, glutarnergic, adrenergic, dopaminergic, GABAergic and serotonergic receptor function in the cerebral cortex. Thus the results suggest the rejuvenation of brain function by increased neural plasticity and activity as a function of age.

15. Serum T3 concentration was decreased in STH treated young and old rats compared to saline treated young and old rats. In insulin treated young and old rats the content was reversed to near control. Our results suggest that the balanced metabolic hormones make the cellular function more efficient.

16. Calcium imaging results showed that carbachol, the cholinergic agonist, increased the Ca^{2+} release from the pancreatic islet cells of young and old rats in vitro. Stimulatory effect of carbachol on Ca^{2+} release was mediated through muscarinic M1 receptors in young and old rats.

17. A prominent brain activity difference was observed in diabetic rats when compared to control rats by EEG analysis. Long term low dose insulin and STH treatment found to be pronounced in brain wave signaling pattern in old rats. This
will have tremendous importance in improving memory, cognitive impairment and rejuvenating brain functions on ageing.

It is evident from our results that brain muscarinic M1 and M3 receptor functional balance plays a major role in cholinergic functional regulation during diabetes as a function of age. Gene expression studies of muscarinic M1 and M3 receptors showed a prominent cholinergic functional difference in brain regions of diabetic rats. In vitro studies confirmed the regulatory role of acetylcholine, muscarinic M1 and M3 receptor subtypes in IP3 and cGMP release by pancreatic islets. These findings have important implications for understanding the molecular mechanisms underlying memory and cognitive impairment by second messengers due to diabetes and ageing. A reduced secretion of thyroid hormones with age was observed, which is an indicative of an age-related impairment in metabolic and neurological functions. Calcium imaging studies revealed a prominent role of muscarinic M1 mediated Ca\(^{2+}\) release from the pancreatic islet cells of young and old rats. Electrophysiological studies using EEG recording showed disturbed brain activity during diabetes in old rats compared to young. Long term low dose STH and INS administration found to be effective in neurotransmitter receptor functional regulation in improving memory, cognitive impairment and rejuvenating brain functions.

The functional improvement of muscarinic M1, M3, glutamate NMDAR1, mGlu-5, \(\alpha_{2A}, \beta_{2}, \text{GABA}_{A_{2}}, \text{GABA}_{B}, \text{DAD2}\) and \(5-\text{HT}_{2C}\) receptors mediated through IP3 and cGMP will lead to therapeutic applications in the management of diabetes. Also, long term low dose STH and INS treatment improved brain function which has clinical significance in managing functional deterioration during ageing.
Conclusion

We conclude from our studies that acetylcholine through muscarinic M1 and M3 receptors play an important role in the brain function during diabetes as a function of age. Cholinergic activity as indicated by acetylcholine esterase, a marker for cholinergic function, decreased in the brain regions - the cerebral cortex, brainstem and corpus striatum of old rats compared to young rats. In diabetic condition, it was increased in both young and old rats in cerebral cortex, and corpus striatum while in brainstem it was decreased. The functional changes in the muscarinic receptors were studied in the brain regions and it showed that muscarinic M1 receptors of old rats were down regulated in cerebral cortex while in corpus striatum and brainstem it was up regulated. Muscarinic M3 receptors of old rats showed no significant change in cerebral cortex while in corpus striatum and brainstem muscarinic receptors were down regulated. During diabetes, muscarinic M1 receptors were down regulated in cerebral cortex and brainstem of young rats while in corpus striatum they were up regulated. In old rats, M1 receptors were up regulated in cerebral cortex, corpus striatum and in brainstem they were down regulated. Muscarinic M3 receptors were up regulated in cerebral cortex and brainstem of young rats while in corpus striatum they were down regulated. In old rats, muscarinic M1 receptors were up regulated in cerebral cortex, corpus striatum and brainstem. In insulin treated diabetic rats the activity of the receptors were reversed to near control. Pancreatic muscarinic M3 receptor activity increased in the pancreas of both young and old rats during diabetes. In vitro studies using carbachol and antagonists for muscarinic M1 and M3 receptor subtypes confirmed the specific receptor mediated neurotransmitter changes during diabetes. Calcium imaging studies revealed muscarinic M1 mediated Ca\(^{2+}\) release from the pancreatic islet cells of young and old rats. Electrophysiological studies using EEG recording in young and old rats showed a brain activity difference during
diabetes. Long term low dose STH and INS treated rat brain tissues were used for gene expression of muscarinic M1, M3, glutamate NMDAR1, mGlu-5, α2A, β2, GABA_Aa1 and GABA_B, DAD2 and 5-HT_2C receptors to observe the neurotransmitter receptor functional interrelationship for integrating memory, cognition and rejuvenating brain functions in young and old. Studies on neurotransmitter receptor interaction pathways and gene expression regulation by second messengers like IP3 and cGMP in turn will lead to the development of therapeutic agents to manage diabetes and brain activity.

Thus from our results it is suggested that functional improvement of muscarinic M1, M3, glutamate NMDAR1, mGlu-5, α2A, β2, GABA_Aa1 and GABA_B, DAD2 and 5-HT_2C receptors mediated through IP3 and cGMP will lead to therapeutic applications in the management of diabetes. Also, our results from long term low dose STH and INS treatment showed rejuvenation of the brain function which has clinical significance in maintaining healthy period of life as a function of age.