Summary and Conclusion

Memory loss is one of the most common features in various neurological disorders and physiological state of aging. This memory loss or amnesia is associated with socio-economic burden as it impairs not only the normal daily activities of the affected individuals but also their families and caregivers. Therefore, knowledge of the molecular mechanisms that regulate the process of learning and memory is essential to clarify the etiology of pathological amnesia as well as to develop therapeutic approaches. To study memory, its impairment and the effect of various psychoactive drugs, researchers have commonly used animal models with disrupted cholinergic neurotransmission. One such potent psychoactive drug is scopolamine derived from the plants of Solanaceae family like Belladonna, Mandrake, Datura, etc. It is an alkaloid non-selective muscarinic receptor antagonist that blocks the effects of acetylcholine, impairs LTP and induces amnesia in rodents.

Several evidences from our lab and others show alterations in molecular events leading to memory loss. Studies have shown that plasticity genes such as BDNF, GFAP, Arc, Egr1, Homer, Narp and KLK8 are downregulated, while epigenetic regulators HDAC2 and DNMTs are increased in scopolamine-induced amnesia. Thus, these plasticity marker genes play an important role in the process of memory formation and alteration in their expression causes synaptic dysfunction and memory impairment.

These evidences suggest that scopolamine-induced amnesia is a well-established model. However, the major lacuna lies in molecular details and the role of signaling pathways that underlie its amnesic action. Majority of studies have linked it with cholinergic blockade and few studies suggest that it might also influence genes involved in metabolism, neuronal apoptosis, cytoskeleton reconstruction, protein trafficking and cell differentiation during amnesia. However, muscarinic signaling and synaptic plasticity might not be solely responsible for amnesic effect of scopolamine and other pathways could crucially contribute for the memory impairment. Therefore, we have analysed the hippocampal proteome of scopolamine-induced amnesic mice and identified proteins which showed alterations in their expression.

In the first chapter, we have generated the scopolamine-induced amnesic mouse model by treating Swiss albino male mice (10±2 weeks) with standardized dose (3 mg/kgbw) intraperitoneally for 7 consecutive days. Novel object recognition test showed significant impairment in learning and memory of scopolamine administered mice. Thus, our study
provided behavioral validation for memory impairment in scopolamine-induced amnesic mice. After generation of amnesic mouse model and its validation, we analysed the hippocampal proteome using 2-DE coupled with MALDI-TOF/MS analysis. We identified 18 proteins which showed alteration in their expression. Out of these proteins, 11 were downregulated and 7 were upregulated in the amnesic mice as compared to control. Then these proteomic results were validated by qRT-PCR and western blotting. Thereafter, we selected five most downregulated and five most upregulated proteins for the analysis of mRNA level using gene specific primers and found similar expression pattern as observed in proteomic study. Then, we selected two proteins which were maximally downregulated (Vdac1) and upregulated (Coronin 1b) for validation by western blotting at protein level. Similar expression pattern was observed in western blotting also. Further, the identified proteins were classified on the basis of their Gene Ontological functions including biological, molecular, cellular component and protein class through biocomputational tool. The majority of proteins among biological processes are involved in metabolic process (47.10%), among molecular functions in catalytic activity (58.3%), among cellular components in cell part (44.4%), and among protein classes in cytoskeleton (20%) and transferase (20%). Further, functional pathway analysis was performed and different pathways were predicted for the identified proteins. Then interaction network of the identified proteins was explored and interestingly we observed that majority of proteins including Fascin 1 and Coronin 1b showed common association with Actg1 cytoskeleton and Vdac1 transporter protein.

In the second chapter, we selected Vdac1 for further study because it was maximally downregulated and showed common interaction with other proteins. We checked the downstream function of Vdac1 during scopolamine-induced amnesia. As Vdac1 is involved in energy metabolite transportation, we measured total ATP level in the hippocampus of amnesic mice using Luciferase assay. Total ATP level was decreased in the hippocampus of scopolamine-induced amnesic mice as compared to control. The depletion in ATP level caused disruption in the mitochondrial membrane potential, ultra-structure, and increased the deposition of calcium ions in the mitochondria of scopolamine-induced amnesic mice. These remarkable changes in the mitochondria cause mitochondrial dysfunction. Furthermore, ROS production was elevated and SOD and catalase enzyme activity was reduced. The loss of mitochondrial membrane integrity caused the release of cytochrome c from membrane to cytosol as assessed by western blotting in cytosolic fraction of hippocampus of scopolamine-induced mice. Then, we checked the expression of different
apoptotic marker proteins (Bax, Bad, Casp3). The expression of pro-apoptotic proteins was upregulated in the hippocampus of amnesic mice as compared to control. Lastly, apoptosis level was checked at cellular level using FJC staining. The number of degenerated neuronal cells was increased in subregions (DG, CA3, CA1) of hippocampus of scopolamine-induced amnesic mice. These findings suggest that downregulation of Vdac1 might cause ATP depletion, mitochondrial disintegration, apoptosis and neurodegeneration in the hippocampus of scopolamine treated mice.

In third chapter, we silenced the expression of Vdac1 in the hippocampus of normal young mice to confirm the involvement of Vdac1 in mitochondrial function, neurodegeneration and learning and memory. The expression of Vdac1 was decreased in siRNA infused mice as compared to negative control. Further, total ATP level was found to decrease leading to disruption of the mitochondrial membrane potential in Vdac1 silenced mice. Thereafter, we checked the neurodegeneration and recognition memory. The number of degenerated neuronal cells was increased in all subregions of hippocampus of Vdac1 silenced mice. Vdac1 siRNA infused mice also showed impairment in recognition memory. Thus, these findings suggest that downregulation of Vdac1 is involved in mitochondrial disintegration and neurodegeneration leading to memory impairment.

In conclusion, our study demonstrated that during scopolamine-induced amnesia, hippocampal proteome shows differential changes and the altered proteins are involved in diverse functions such as cell metabolism, catalytic activity, chaperone and cytoskeleton architecture. Among all the altered proteins, the energy transporter protein Vdac1 was maximally downregulated and showed interaction with many other proteins, suggesting its role as a hub protein. The downregulation of the energy transporter protein Vdac1 and impairment of its downstream function resulted in the depletion of ATP level, mitochondrial disintegration and increased number of degenerated neuronal cells in the hippocampus of scopolamine-induced amnesic mice. Further, hippocampal Vdac1-silencing in normal young mice showed reduction in ATP level and mitochondrial membrane potential, which in turn increased the number of degenerated neuronal cells. Thus, our findings suggest that downregulation of Vdac1 is associated with ATP depletion and mitochondrial disintegration leading to hippocampal neurodegeneration and impairment of recognition memory in Vdac1 silenced mice as depicted in Fig. 26.
Fig. 26. Proposed mechanism for altered energy metabolic pathway in the hippocampus of scopolamine-induced amnesic mice: Vdac1 downregulation causes depletion of ATP level, mitochondrial disintegration, ROS elevation, and reduction in antioxidant enzyme activity leading to apoptosis, neurodegeneration and impairment in recognition memory in the hippocampus of scopolamine-induced amnesic and Vdac1-silenced mice.