Aging is viewed as a multifactorial process and even with a well-described definition and a familiar set of characteristics, aging remains one of the most poorly understood of all biological phenomena, due in large part to its inherently complex and integrative nature, as well as the difficulty in dissociating the effects of normal aging from those manifested as a consequence of age-associated disease conditions. Aging is associated with increased susceptibility to neuronal loss and disruption of cerebral function, either as a component of senescence, or as a consequence of neurodegenerative diseases or stroke. Age-related neuronal dysfunction causes a decline in cognitive function and other subtle changes within the cortex including alterations in receptors, loss of dendrites, spines and myelin dystrophy, as well as the alterations in synaptic transmission. Changes in multiple aspects of neurotransmitter signaling, for example, declines in the levels of neurotransmitters such as acetylcholine are well documented in the aging brain. Similarly, oxidative stress is ubiquitously observed hallmark of neurodegenerative disorders. Neuronal cell dysfunction and cell death due to oxidative stress may causally contribute to the pathogenesis of progressive neurodegenerative disorders. Thus, a serious loss of cholinergic function in the central nervous system contributes to cognitive symptoms during aging and these changes in vulnerable populations of neurons are exacerbated in neurodegenerative disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), and amyotrophic lateral sclerosis (ALS).

Acetylcholinesterase (AChE; EC 3.1.1.7) is an enzyme working at the neuronal synapse to quench or hydrolyze the chemical signal transmitter, acetylcholine (ACh), which is released from the presynaptic nerve terminal in response to an external electrical impulse, called an action potential. AChE has evolved into one of the fastest enzymes because ACh must be degraded rapidly to restore the synapse to the initial phase for receiving the next neural signal transmission. AChE is expressed in a variety of tissues including muscle, nerve, white and grey matter of brain, in amniotic fluid, placenta and hematopoietic cells. AChE has been hypothesized to play a variety of non-cholinergic and even non-catalytic roles in embryos and adults. AChE has been used as a marker for cholinergic function in neural tissue because of its implications in synaptogenesis and its involvement in neurodegenerative diseases like Alzheimer’s disease and cancer.
Perhaps the best known case of lifespan plasticity is the dietary restriction (DR) response. Dietary restriction has several beneficial effects on physiological processes viz., carbohydrate, protein and amino acid metabolism; immunological and neuroendocrinological signaling systems. It can change protein turnover, modifies the expression of many genes and decreases glycation of cellular and extracellular macromolecules. Similarly, understanding the complex and integrative interactions of different hormones and neurotransmitters of the endocrine system pave a way for hormone replacement paradigms with the intention to restore a youthful hormone profile and therefore reverse the clinical signs and symptoms of aging, the focus of many endocrine studies of aging.

Keeping in mind about the different classical and non-classical roles of AChE during the developmental, reproductive, aging processes and in wide array of physiological and patho-physiological diseased conditions, the work embodied in this thesis was conceived with the following objectives:

i. Assessment of endogenous activity level of AChE in different tissues which includes brain (cerebral hemispheres, cerebellum), liver, kidney, heart and spleen of female mice at different postnatal ages i.e. at 1-, 6- and 18-month old.

ii. To determine the effect of different stress hormones like dexamethasone (synthetic glucocorticoid hormone), epinephrine, norepinephrine and dopamine (catecholamines) on the activity of AChE in different tissues of mice as a function of age.

iii. To confirm that if any, modulation in AChE activity is due to the direct effect of the above different hormones under study, by blocking of the individual hormone with their specific receptor antagonist.

iv. To determine the effect of short-term dietary restriction (24 h fasting and 24 h re-feeding) on the AChE activity in different tissues under study as a function of age.

v. To determine the effect of long-term dietary restriction (3-months alternate day feeding and non-feeding regimen) on AChE activity in different tissues as a function of age.
To ascertain protein levels of AChE by slot as well as Western blot analyses. This was conceived to contemplate the changes, if any, at the level of AChE protein expression.

**ENDOGENOUS LEVEL OF AChE AS A FUNCTION OF AGE**

Our findings reveal that AChE exhibits a different pattern in its specific activity which is dictated in an age- and tissue- specific manner. Amongst the regions of the brain and different tissues studied, the highest level of AChE activity was found in the cerebral hemispheres, followed by the cerebellum, liver, spleen, heart and kidney. Representative data also showed age- dependency in AChE activity and its protein expression pattern in different tissues, with increased level seen in the cerebral hemispheres, kidney and heart of 1-month young as compared to the 18- month aged mice. On the contrary, in the spleen a higher level of AChE activity was observed in the aged in comparison to the young ones. However, AChE activity and its protein expression level remained the same in the cerebellum and liver of all the three ages. High level of AChE in young mice indicate higher metabolism of acetylcholine associated with learning and/or exploratory, neuronal differentiation or cellular maturation processes, intercellular and intracellular regulatory mechanisms besides its several non–classical functions like regulation of cell growth and cell adhesion. Alternatively, the decline in the activity level of this enzyme in old age may be due to alterations in the template activity of corresponding genes that may be brought about by various effectors or modulators produced during growth, gradual loss of neurons or a decrease in the rate of AChE protein synthesis itself.

**HORMONAL REGULATION OF AChE AS A FUNCTION OF AGE**

Our studies showed that dexamethasone significantly increased the activity of AChE in all the tissues except in the cerebral hemispheres and cerebellum. The magnitude of this induction is dictated in an age- and tissue- specific manner. The antagonizing effect of RU486 on dexamethasone modulation of AChE activity was also studied in the brain (cerebral hemispheres, cerebellum), liver, spleen, heart and kidney. RU486 significantly antagonized the dexamethasone effect in the above mentioned tissues by decreasing the activity of AChE in an age- and tissue- specific
manner. Western and slot blotting confirmed all the above changes observed at the level of AChe protein. These findings clearly demonstrated the fact that the observed increase in AChe activity by dexamethasone is mediated through its binding and signaling through glucocorticoid receptor and that the varied effects indicate the ‘permissive role’ of glucocorticoids or tissue specific action of this hormone, which in turn depends upon the tissue level of glucocorticoid receptors. Similarly, an independent non-classical action of glucocorticoids, the unique sensitivity, selectivity and metabolism of dexamethasone cannot be ruled out for the possible observed varied effects.

Epinephrine significantly increased the activity of AChe in liver, kidney, spleen and heart of both young (1-) and aged (18- month) mice. However, no statistical significant changes were observed in cerebral hemispheres and cerebellum of both young and aged mice in comparison with the respective age-matched control group. The degree/magnitude of epinephrine effect in the mentioned tissues is dictated in an age- and tissue- specific manner. Our result also indicates that propranolol significantly antagonized the observed epinephrine effect by decreasing the activity of AChe in liver, kidney and heart of both young (1-) and aged (18- month) mice. Phentolamine significantly antagonized the observed epinephrine effect by decreasing the activity of AChe in the heart of both young and aged mice. Western and slot blotting confirmed all the above changes observed at the level of AChe protein.

Norepinephrine significantly decreased the activity of AChe in kidney and heart of mice and this is dictated in an age- and tissue- specific manner. However, no significant changes were observed in the cerebral hemispheres, cerebellum, liver and spleen upon treatment in both young and aged mice as compared to the respective age-matched control group. Western and slot blotting confirmed all the above changes observed at the level of AChe protein. Our results also indicate that dopamine significantly increased the activity of AChe in liver and kidney of mice and this effect shows an age-specificity. On the contrary, it was also observed that dopamine produced an inhibitory effect in the spleen of young (1- month) mice. However, no statistical significant changes were observed in cerebral hemispheres, cerebellum and heart of both young and aged mice in comparison with the respective age-matched control group.
EFFECT OF SHORT-TERM DIETARY RESTRICTION (24 h FASTING AND RE-FEEDING) ON AChE ACTIVITY

Our result showed that on 24 h fasting, the level of AChE activity decreased significantly in the cerebral hemispheres and heart of 1-month young mice as compared to the respective *ad libitum* fed control. Re-feeding the mice for another 24 h after fasting reversed the above observed changes. However, no significant changes were observed either during 24 h of fasting or re-feeding in any other tissues under study in both the ages studied. The present study of short-term dietary restriction indicates the level of high synaptic plasticity of neurons present in cerebral hemispheres and heart of young mice and that diet plays an important role in its regulation through AChE during maturation process.

EFFECT OF LONG-TERM DIETARY RESTRICTION ON AChE ACTIVITY

We have also studied the effect of long-term DR (three months regimen by alternate day of feeding and non-feeding) in 1-month young and 18-month aged mice. The present study reveals that after three months of food restriction, there was significant body weight reduction in both young and aged mice. Irrespective of the day of sacrificing the animals i.e. on the day of feeding and non-feeding day, long-term DR decreased AChE activity and its protein expression level in most tissues of 1-month young and 18-month aged. These findings indicate that long-term DR has a more pronounced and cumulative effect in reducing AChE activity. It further support the importance of long-term dietary restriction as potential AChE inhibitor which may pave the way in controlling different AChE related pathophysiological conditions.
From the findings embodied in this thesis, it is concluded that:

- AChE and its protein level express in a tissue- and age- specific manner reflecting the different role of AChE at the different phase in the lifespan of an organism.
- Dexamethasone treatment increased AChE activity and its protein expression level by binding to glucocorticoid receptor and this regulation is based upon the tissue sensitivity and age of the mice.
- Epinephrine treatment increased AChE activity and its protein expression level in an age-and tissue- specific manner. This effect is manifested through its binding to β-adrenergic receptors in most tissues and in combination with α-adrenergic receptor in the heart.
- Norepinephrine treatment decreased AChE activity and its protein expression level in kidney of 1-month young and 18-month aged mice. Similarly, in the heart a decrease in 1-month young mice and remained constant in 18-month aged mice was observed upon norepinephrine treatment. A significant antagonistic effect of propranolol in comparison with the age-matched group that received only norepinephrine treatment was observed only in the kidney.
- Dopamine significantly increased the activity of AChE and its protein expression level in liver and kidney of mice and this effect is dictated in an age-specific manner. On the contrary, it was also observed that dopamine produced an inhibitory effect in the spleen of 1-month young mice. The significant antagonistic effect in the presence of haloperidol confirmed that the effect on AChE was due to its binding to dopamine receptors.
- 24 h fasting decreased AChE activity and its protein expression only in the cerebral hemispheres and heart of young mice which is restored after 24 h of re-feeding, indicating that short-term dietary interventions play a role in regulating the activity of this enzyme in post-mitotic organs during maturation process.
- Long-term dietary restriction decreased AChE activity and its protein expression in most tissues studied except cerebellum and spleen. These
findings indicate that long-term DR have a more pronounced and cumulative effect on AChE, thereby, confirming the benefit of long-term DR as lifestyle AChE inhibitor versus synthetic drugs.

The findings from the present study provide an insight into the basic role of AChE during the process of aging in mice. It also enlightened the complex and integrative interactions of different hormones in aging process which can be useful as AChE activators and/ or inhibitors in different pathophysiological conditions. Furthermore, it brought forward the importance of diet in cholinergic function and that dietary restriction can be adapted as lifestyle AChE inhibitor to prevent age-related neurodegerative diseases.