SUMMARY

Glucocorticoids play a key role in regulating a wide variety of biological responses. Virtually all tissues in the body are target organs for glucocorticoids and can respond in one way or another (Borski 2000). In spite of the broad spectrum of biological effects induced by glucocorticoids, there is a set mechanism whereby target cells can respond to the hormonal signal, through the mediation of a receptor protein called glucocorticoid receptor (GR) in most target cells (Carlstedt-Duke 1999). Now it is known that there is a single gene for GR in all vertebrates. Thus, there must be other mechanisms that modulate the action of GR to enable the myriad roles of glucocorticoid actions in animals, including humans. A number of reports indicate the presence of tissue-specific factors/proteins/enzymes that may specify and/or modulate the cellular role of GCs through GR (Bamberger et al 1996). These factors may act at a particular or distinct step of steroid action. A number of such factors such as pyridoxal 5-phosphate, free fatty acids, biotin, melatonin, parathymosin etc have been attributed under physiological conditions to regulate GR action. Apart from these, a number of signal molecules and tissue-specific transcriptional co-activators/co-repressors have also been demonstrated to regulate the GR mediated gene expression in target cells (Tronche et al 1998). Hence, GR action can be modulated by a number of factors, which may alter its tissue responsiveness. In view of the diverse role of GR and the susceptibility of its regulation by various agents, it was decided to study the modulatory effects of some endogenous and exogenous agents on hormone binding to receptor and its stabilization, activation, DNA binding and also the effects of diabetic state and senescence onto GR.

Findings of work done are summarized below under various sections:

Steroid binding to GR and stabilization of steroid-bound receptor

Dithiothreitol (DTT) significantly increased (maximally at 4-8 hr) hormone binding to hepatic GR compared to control. However, DTT did not show any significant increase in stabilization of hepatic hormone-bound GR. Mercaptoethanol and glutathione were ineffective in enhancing the steroid binding to hepatic GR compared to control, with no influence on hormone-bound receptor stabilization. Furthermore, none of these reducing agents could show any differences in steroid binding to GR and the stabilization of hormone-bound receptor in an age- and tissue-specific manner.
Activation modulation of GR

Both heat (25°C) as well as salt (20 mM Ca\(^{2+}\)) were able to activate the GR from the liver and kidney of mice. Interestingly, the activation of GR by heat is more pronounced in mature (120-day) animal's liver as compared to immature (15-day), without any such changes in the case of kidneys. This difference in heat activation of immature and mature hepatic GR was attributed to alterations in the receptor property as evident from cross-mixing experiments. Various exogenous and endogenous agents used to modulate the activation of receptor did modulate the activation process. Cadmium, selenite and arsenite were found to be inhibitors of heat activation of GR in liver and kidney. The potency of these modulators was cadmium > selenite > arsenite. Additionally, leupeptin and PUFAs were also found to be potent inhibitors of heat activation of GR. Among the PUFAs, both linoleic and arachidonic acid had greater potency (~70% at 160 μM) in inhibiting GR heat activation compared to oleic acid (38% at 40 μM). Interestingly, pyrophosphate (PPi), unlike other modulators, was found to significantly induce (~65%) activation of GR at 0°C from both liver and kidney. However, the magnitude of activation modulation by these modulators remains the same at the two ages studied, indicating that the mechanism(s) of activation modulation does not get altered during these ages of mice.

Acceptor binding modulation of GR

Various modulators of activated receptor binding to DNA inhibited the binding of GR to DNA. Pyridoxal phosphate (PLP) was found to be a potent inhibitor of activated receptor binding to DNA. Besides PLP as a physiological inhibitor of activated receptor binding to DNA, aurintricarboxylic acid (ATA), a synthetic triphenylmethane dye, and methyl methanethiosulfonate (MMTS) were also found to be effective inhibitors of GR binding to DNA. Among these, ATA exhibits strong inhibition followed by PLP and MMTS as evident from their IC\(_{50}\) values. These modulators do not yield any age- and tissue- specificity in inhibiting the activated receptor binding to DNA-cellulose and nuclear DNA.

Diabetes and GR modulation

STZ-induced diabetic mice exhibited a similar level of GR in the liver and kidney of immature (15-) and mature (120-day) animal as compared to control, without any change in the affinity (K\(_{d}\)) for the hormone. This shows that STZ-induced diabetes have no effect on modulating the level of GR and the affinity for the hormone in either liver or kidney at these two ages studied. However, STZ-induced diabetes decreased the heat activation of hepatic GR from diabetic animals in both the ages studied, with no such decrease in the kidney, thereby indicating tissue- specificity. Such decrease in activation of hepatic GR in diabetic mice is attributed to receptor specificity as judged by cross-mixing experiments. These observations indicate that
the reduced hepatic GR activation during STZ-induced diabetes might play an important role in controlling glucose homeostasis in diabetic animals.

**Aging and GR modulation**

Aging and GR modulation studies have indicated changes in GR concentration, heat activation, activation modulation by PUFAs and chromatin organization during old age of mice. The level of GR is significantly reduced in the liver and kidney of older (120-week) mice as compared to young (4-week) ones, however, with no change in the affinity ($K_d$) for the hormone. Also, the magnitude of heat activation of GR was more pronounced in the liver and kidney of young mice than those from older ones. Polyunsaturated fatty acids (PUFAs), linoleic and arachidonic acid showed variable impact on activation inhibition of GR in an age-specific manner. Linoleic acid caused greater inhibition of GR heat activation in the liver and kidney of young mice as compared to old ones. Whereas, arachidonic acid exhibited greater inhibition of GR activation only in the liver of young mice as compared to old. In contrast, the inhibition of renal GR heat activation by arachidonic acid was age-independent. DNase I digestion of hepatic and renal nuclei from young and aged mice revealed significant higher digestion extraction of bound GR complexes from young animal tissues compared to old ones. These findings indicate more compact nuclear chromatin organization in old mice's tissues. Such alterations may contribute towards functional changes in glucocorticoid action and responsiveness in target tissues of senescent animals.

In conclusion, the findings summarized in this thesis indicate glucocorticoid receptor modulation by various endogenous/exogenous modulators and also by diabetic state and old age in mice. Such modulation of GR may in turn be responsible for tissue's responsiveness towards glucocorticoids during animal's health and diseases.