Summary

BIOCHEMICAL INVESTIGATIONS ON CARDIOVASCULAR FUNCTIONS IN DIABETES

SUMMARY OF THE THESIS

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More than 20 to 30 million people throughout the world suffer from diabetes mellitus. Diabetes mellitus is a clinical expression of absolute or relative insulin deficiency. Oral hypoglycemic drug or insulin therapy can lower the blood glucose level in diabetics but can not rectify secondary complications. Amongst all secondary complications, effect on cardiovascular system is maximum. Overall, 66 % of all morbidity and mortality in diabetes is due to cardiovascular disease. The incidences of coronary heart disease (CHD) and congestive heart failure (CHF) are higher in the human diabetes females compared to the human diabetic males. The classical cardiovascular risk factors can not account for all excess risk of cardiovascular disease associated with diabetes. The underlying biochemical defects, however, are not clearly delineated.

The butyrylcholinesterase (BChE) activity is about 85 % of the total cardiac cholinesterase activity, while acetylcholinesterase (AChE) activity is about 15 %. Interestingly the physiologic hydrolysis of acetylcholine in heart is brought about by AChE. The function of BChE in heart is unclear. Earlier workers have shown that plasma BChE activity increases in diabetic rats; the increase in the activity was significantly higher in female rats than in the male rats.

Membrane structure function alterations play important role in the secondary complications of diabetes. In view of the

above properties of AChE and Na^+,K^+ ATPase, and lipid/phospholipid composition of erythrocyte membranes were monitored as markers for membrane function. Simultaneously the BChE activity in the serum was also determined. These studies were carried out in humans and experimental animals i.e. rats.

Since it was not possible to carry out studies on the role of cholinesterase in heart of diabetic patients in relation to increased incidences of CHD and CHF, alloxan-diabetic rat was used as a model system. Kinetic properties of soluble and membrane-bound form of cardiac BChE were studied in both diabetic and insulin-treated diabetic rats.

For human studies, the volunteers were grouped in four groups

a) non-diabetic control (Control)
b) untreated diabetic (UTD)
c) insulin-treated diabetic (ITD) and
d) tablet (oral hypoglycemic drug, sulfonylurea) treated diabetic (TTD).

In humans insulin and tablet treatment were able to control elevated blood sugar level but were not able to control elevated erythrocyte membrane protein glycosylation. Decreased erythrocyte membrane Na^+,K^+ ATPase activity in the diabetics could be restored partly by insulin treatment but not by tablet treatment. The opposite was true for serum BChE

activity. The Km and Vmax of the components of erythrocyte membrane Na^+,K^+ ATPase were differentially affected. In insulin treated diabetics component II of serum BChE was absent. In vitro incubation with insulin had differential effects on erythrocyte membrane Na^+,K^+ ATPase and serum BChE; AChE activity was not significantly influenced.

In the diabetic condition the total phospholipid (TPL) content decreased and cholesterol (CHL) content increased in erythrocyte membranes, and no treatment was able to impair the condition. As far as human erythrocyte membrane phospholipid composition is concerned no therapy was able to restore alterations caused by diabetes and the situation worsened after treatment. The membrane fluidity decreased in diabetes and insulin treatment resulted in significant fluidization of the membrane. The temperature kinetics of AChE did not change in both the treatment conditions. In case of temperature kinetics of serum BChE phase transition was evident in diabetic group unlike control. In some patients insulin was able to rectify the defect but the tablet treatment worsened the situation.

Next set of experiments were carried out with alloxan-diabetic rats as a model. Sex linked differences were observed in blood sugar level and daily urinary sugar excretion of the alloxan diabetic male and female rats. The restoration of body weight and control of blood sugar level was less efficient in the insulin treated diabetic female

rats. Sex-dependent changes were also noted in erythrocyte membrane and serum protein glycosylation. The erythrocyte membrane TPL content decreased in diabetic females, while CHL content increased. Insulin treatment showed differential effects on erythrocyte membrane TPL and CHL content in both male and female rats. Diabetic state resulted in decreased acidic phospholipid content in the males, but extent of decrease was substantially high in the females. Insulin treatment was able to restore levels of acidic phospholipids. In diabetic males the erythrocyte membrane became more rigid, while opposite was true for the females.

In diabetic male rats erythrocyte membrane Na^+,K^+ ATPase activity increased, while it was found to be decreased in diabetic female rats. One component of Na^+,K^+ ATPase was present in erythrocyte membrane of male rats, while two were present in the females. Na^+,K^+ ATPase components were differentially affected after insulin treatment in rats of both the sexes. AChE activity was not detectable in both diabetic as well as insulin treated diabetic group of animals of both the sexes. The Arrhenius plots for erythrocyte membrane AChE in females was opposite to that of the male rats. An 18^oC difference was observed in phase transition temperature of AChE in both the sexes.

Serum BChE activity was higher in the control female rats than in the males. Serum BChE activity increased only in the

male diabetic rats but not in female diabetic rats. Insulin treatment had no effect on serum BChE activity. In diabetic male rats Vmax of both the components of serum BChE increased, while insulin treatment showed no effect on serum BChE substrate kinetics in female rats. Decrease in energy of activation E1 and E2 was observed in diabetic male and female rats respectively. Insulin treatment was able to rectify defect in serum BChE in terms of temperature kinetics.

In the diabetic male rats there was a proportionate decrease in heart weight and body weight; and insulin treatment brought about a proportionate restoration. In control females soluble and membrane-bound cardiac BChE activity was very high than in the control males. The soluble BChE activity increased in the males but decreased in females. Insulin treatment resulted in substantial increase in the activity in both males and females. In diabetic state cardiac membrane-bound BChE activity increased in animals of both the sexes. Km of two components of soluble cardiac BChE decreased in diabetic condition, which continued even after insulin treatment in both male and female rats. Similar pattern was observed for membrane-bound form of cardiac BChE in the male rats, while in the females Km of component I increased in diabetic and insulin treated diabetic rats.

Temperature kinetic of soluble and membrane-bound cardiac BChE was affected differentially in diabetic condition in rats from both the sexes. Differential effects of insulin

treatment was not noted on soluble and membrane-bound cardiac BChE in rats from both the sexes.

In conclusion, the present studies have tried to bring into focus the basic biochemical defects in the cardiovascular functions in diabetes in human as well as in the rats. In particular, it was found that all the functional defects in the diabetic state could not be corrected by insulin treatment. This is consistent with the observations of earlier workers that insulin treatment can control the blood sugar level but can not rectify all the maladies of diabetes.