CHAPTER : 7
Effect of alloxan-diabetes on the substrate kinetics of cardiac butyrylcholinesterase in male and female rats.

Introduction

Individuals with diabetes experience acute complications such as hyperglycemia, ketoacidosis and cerebral edema and after a decade with the disease their risk of developing long-term diabetes complications including microvascular (i.e. retinopathy, nephropathy and neuropathy) and macrovascular (i.e. cardiovascular, cerebrovascular and peripheral vascular disease) disorders becomes significant (1). Secondary complications of diabetes involve a number of organ systems but none is more detrimentally affected than the cardiovascular system. Overall 66 % of all mortality and morbidity in diabetes is due to cardiovascular disease (2). The risk of congestive heart failure is higher in females than in male diabetics (3).

The classic cardiovascular risk factors of high blood cholesterol, hypertension and smoking are predictors of cardiovascular mortality, not only in non- diabetic subjects but also in diabetic subjects (4). However, epidemiological data suggest that these classic cardiovascular risk factors do not account for all excess risk of coronary heart disease mortality and morbidity associated with diabetes (5). Cardiac muscle cells in streptozotocin-diabetic rat heart showed condensation of molecular chromatin and folding of nuclear membranes. Swelling of mitochondria, clearing of mitochondrial matrix and incorporation of lysosomal membranes into mitochondrial matrix was noted (6). A marked increase in

both lysosomes and lipid droplets was apparent (6).

The heart in higher vertebrates is one of the tissue containing a large amount of butyrylcholinesterase (BChE). In adult heart of higher vertebrates 85 % of total cholinesterase is BChE and only 15 % is acetylcholinesterase (AChE) (7). Although the activity of BChE in the total atria of the adult rats is in excess compared with AChE, AChE is responsible for the physiological hydrolysis of acetylcholine liberated by the vagus nerve. In contrast, in a more primitive vertebrate, *Torpedo*, BChE is the only cholinesterase present in the heart and is responsible for the physiological hydrolysis of acetylcholine (7).

In diabetic rat, mouse and human serum BChE activity increases (8). One study showed that rise in BChE activity in plasma is not related to the level of BChE production in liver. It has also benn suggested that adipose tissue might be a site of synthesis of BChE (9). The major component of human plasma BChE corresponds to tetramers, composed of disulfide links dimers (10). Non-denaturing electrophoresis ravels the presence of monomers (called C1 in this system) and dimers (C3) in addition to the tetramers (C4) (11). The C2 component has been shown to result from the covalent association of a BChE monomer with albumin (12). The C5 component is a noncovelent conjugate with another as yet unidentified protein (13, 14), while dimer constitutes the only form of BChE in chicken heart (15).

Keeping all the above information in the mind it was planned to measure BChE activity in the soluble and membrane-bound fractions of heart in diabetic and insulin-treated diabetic rats of both the sexes. Studies were also extended to find out the substrate kinetics of these two forms of enzyme in groups described above.

Materials and Methods

Animals were made diabetic as described in Chapter 4 of the thesis (16). Insulin treatment (17) was given to diabetic rats as described in Chapter 4 of the thesis.

Isolation of soluble and membrane-bound forms of BChE

This was achieved essentially by following the procedure of Bisso et al (1991) as described earlier (18, 19). Briefly, the tissue (heart) from male and female rats of all groups was quickly removed after decapitation and placed in beakers containing chilled (0 - 5°C) 38 mM Tris-HCl buffer pH 8.5. The tissue was repeatedly washed with the same buffer and 10 % (w/v) homogenates were prepared using a Potter-Elvehjem type glass-teflon homogenizer. The homogenates were centrifuged at 100,000 x g for 1 h and the supernatant was carefully decanted. The supernatant served as the source of the soluble form of the enzyme. The pellet was resuspended by

gentle homogenization in the same volume of 38 mM Tris-HCl buffer pH 8.5 containing 0.25 % Triton X 100 and subjected to a further centrifugation at 100,000 X g for 1 h. The second supernatant thus obtained was used as the source of the membrane-bound enzyme. All the operations were carried out at $0 - 4^{O}C$.

BChE activity was carried out as described earlier (19, 20) in Chapter 3 of the thesis. Substrate kinetics (21) of BChE was performed as described in chapter 3 of the thesis.

Results

Data in Table 1 show body and heart weights of diabetic and of insulin treated diabetic rats of both the sexes. In the control males the average body weight was 247.8 g, which decreased in diabetic condition by 26 %. After insulin treatment the body weight increased and became comparable to the controls. In control males, the heart weight was 0.672 g which decreased in diabetic condition by 23 % but this decrease was not significant statistically. After insulin-treatment heart weight increased by 30 % and was comparable with the control males. In control male rats, heart weight when expressed as % of body weight was 0.275, which was almost the same in all three groups. Average body weight of control female rats was 243.1 g, which decreased by 29 % in diabetic female rats. After insulin treatment the body weight

Table 1. Effect of alloxan diabetes on body weight and heart weight.

	Control	Diabetic	Insulin treated diabetic
Male			
Body weight (g)	247.8±9.4	182.6±5.9 [a]	245.2±6.4 [#]
Heart weight (g)	0.672±0.077	0.519±0.015	0.676±0.024 [#]
Heart weight % of body weight	0.275±0.003	0.286±0.008	0.279±0.007
Female			
Body weight (g)	243.1±7.2	173.5±6.4 [a]	220.8±10.2 [#]
Heart weight (g)	0.449±0.020	0.463±0.040	0.656±0.015 [a,#]
Heart weight % of body weight	0.211±0.004	0.273±0.011 [a]	0.301±0.015 [a]

Results are expressed as mean±S.E.M. of number of independent observations indicated in the parentheses.

a, $p < 0.001$ compared with corresponding control.

#, $p < 0.001$ compared with corresponding diabetic.

increased by 28 % significantly as compared to the diabetic female rats but was still lower by 9 % as compared to the controls. However, the difference was not statistically significant. While the body weight of the control male rats and female rats were comparable, the heart weight in control female rats was much less i.e. 0.449 g, which was lower by 33 % as compared to the control male rats (Table 1). In diabetic female rats heart weight was comparable with the control females but after insulin-treatment heart weight increased significantly by 46 % and 42 % as compared to the control and diabetic females respectively. In control female rats heart weight expressed as % of body weight was 0.211. In diabetic females it increased by 29 % significantly as compared to the control females. After insulin-treatment the heart weight increased by 10 % as compared to he diabetic female rats although this increase was statistically not significant. Compared to the control females the increase was 42 % which was statistically significant.

Data in Table 2 show BChE activity in soluble fraction of rat heart. In control male rats the activity was 15.8 n mole/min/mg protein, which increased by 186 % in diabetic condition. After insulin treatment the activity increased further significantly by 69 % as compared to the control male rats.

In control female rats the BChE activity was 52.1 n mole/min/mg protein which was 3.3 fold higher as compared to

Table 2. BChE activity in soluble fraction of rat heart.

Animals	Control	Diabetic	Insulin treated diabetic
Male	15.8±1.17(8)	b 45.2±2.04(8)	b,# 76.3±2.10(11)
Female	52.1±2.25(8)	a 41.7±2.24(8)	b,# 73.7±3.23(9)

Results are expressed as mean±S.E.M. of number of independent observations indicated in the parentheses.

BChE activity = n mole / min / mg protein.

a, $p < 0.01$ and b, $p < 0.001$ compared with corresponding control.

#, $p < 0.001$ compared with corresponding control.

the control males. In diabetic condition the activity decreased significantly by 20 %. However, after insulin-treatment the activity increased significantly by 77 % as compared to the diabetic females and was 41 % higher as compared to the control females.

Figure 1 shows typical substrate saturation curves while Figure 2 shows the corresponding typical Eadie-Hofstee plots for substrate kinetics of BChE from soluble fraction of male rat heart. The pattern shows presence of two components of BChE in this fraction (Figure 2). Data in Table 3 give the K_m and V_{max} values derived from substrate kinetics analysis. It can be noted that in the control group the two components of BChE had K_m of 0.31 and 2.3 mM respectively; the two value differed by a factor of 7.3. The V_{max} values were 6.0 and 17.0 n moles/min/mg protein respectively and they differed only in by a factor of 2.7. In diabetic condition K_m of component I decreased significantly by 44 % but the K_m of component II was comparable to the control males. V_{max} of both component I and II increased significantly by 173 % and 211 % respectively. After insulin treatment K_m of component I was still lower as compared to the control values, and now the K_m of component II also decreased significantly by 35 % as compared to both the control and diabetic males. Insulin treatment also resulted in significant increase in the V_{max} of both the components. Thus the V_{max} of component I was 6.5 and 2.4fold higher than the control and diabetic males.
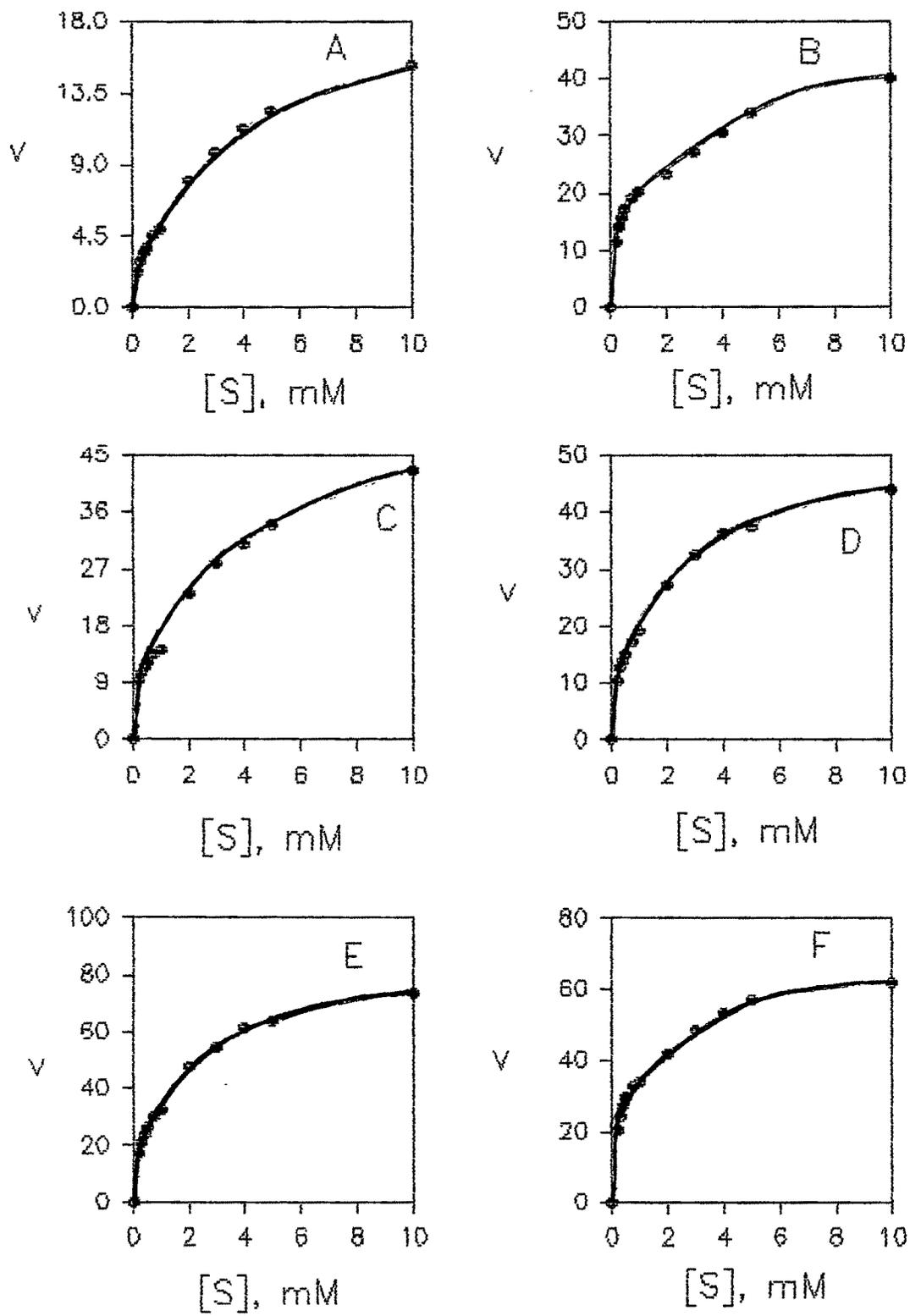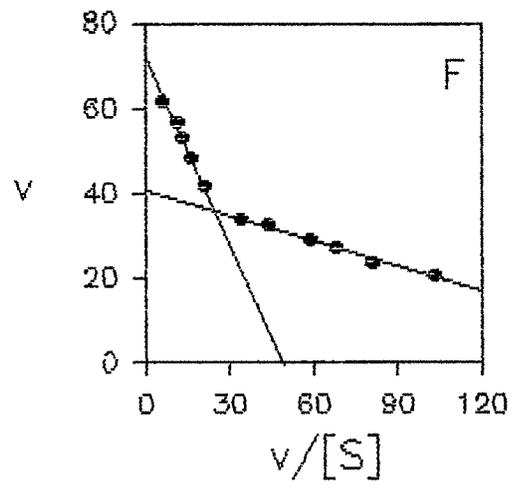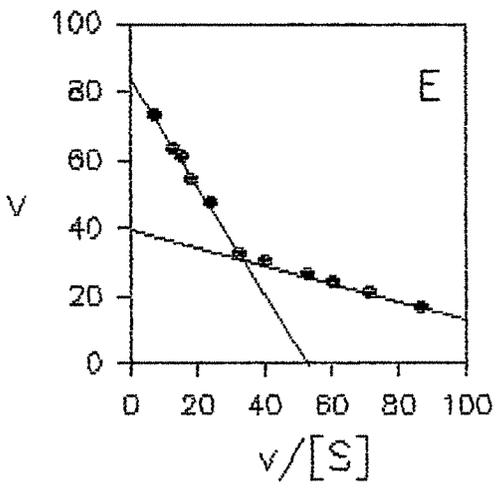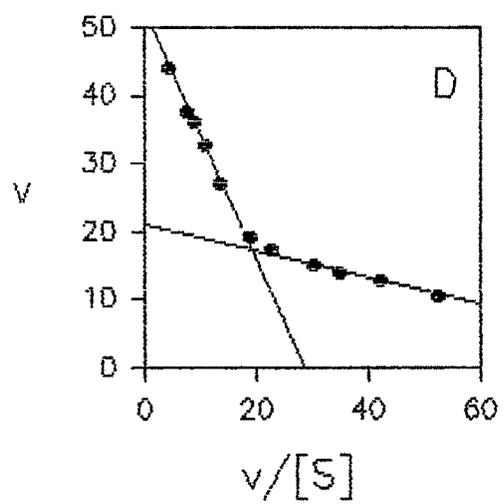
Figure 1.

Figure 2.
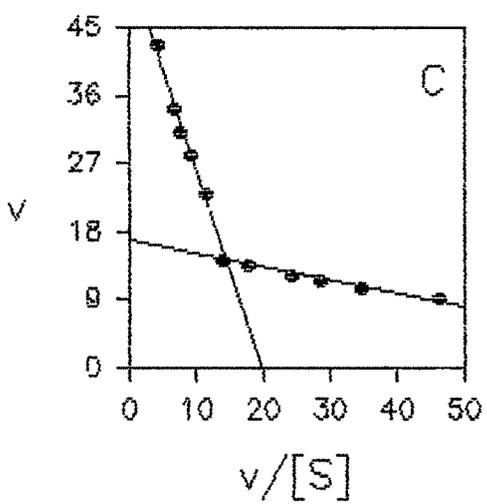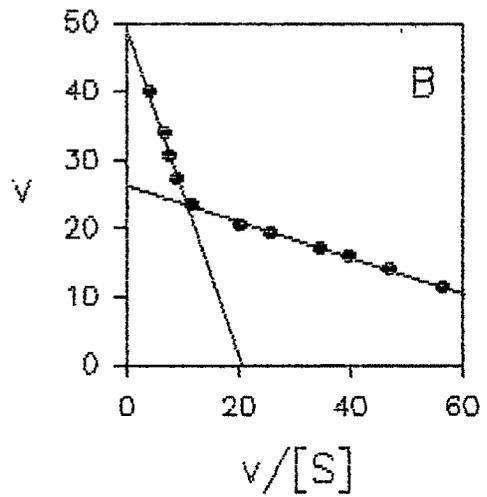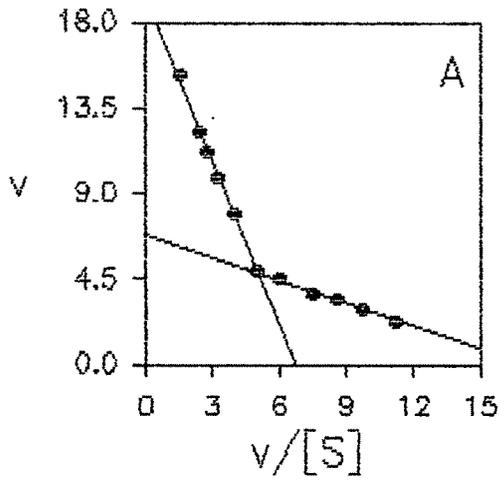
Table 3, Substrate kinetics of soluble form of cardiac in the male rats.

Animals	Component I		Component II	
	Km	Vmax	Km	Vmax
Control (8)	0.31 ± 0.031	6.3 ± 0.33	2.29 ± 0.15	17.0 ± 1.52
	b	b		b
Diabetic (8)	0.17 ± 0.006	17.2 ± 0.94	2.30 ± 0.12	53.1 ± 3.04
	a	b,#	b,#	b,#
Insulin treated diabetic (11)	0.19 ± 0.014	41.4 ± 3.05	1.49 ± 0.07	87.3 ± 2.59

Results are expressed as mean$\pm$S.E.M. of the number of independent observations indicated in the parentheses.

Units : Km = mM; Vmax = n mole / min / mg protein.

a, $p<0.005$ and b, $p<0.001$ compared with corresponding control.

#, $p<0.001$ compared with corresponding diabetic.

Similar trend was seen also for the Vmax of component II. Here the increase in Vmax after insulin-treatment amounted to 5.1 and 1.6 fold compared to the control and diabetic male rats.

Typical substrate saturation curves and Eadie-Hofstee plots for BChE from soluble fraction of female rat heart are also shown in Figures 1 and 2 respectively. Data in Table 4 summarize Km and Vmax values derived from the substrate kinetics data. As is evident (Figure 2), in the female rats also two components of BChE were present in the soluble fraction of heart. Component I was having low Km (0.25 mM) and low Vmax (23.3 n mole/min/mg protein) and component II has high Km (2.2 mM) and high Vmax (57.8 n mole/min/mg protein). In diabetic condition only Km of component II decreased significantly by 21 %; even after insulin treatment Km of component II was the same as in the diabetic group. After insulin treatment Vmax of component I increased significantly by 85 % and 78 % as compared to the control and diabetic females respectively. The same picture was true for Vmax of component II. After insulin treatment Vmax of component II increased significantly by 53 and 71 % as compared to the control and diabetic female rats.

Data in Table 5 show BChE activity from membrane-bound fraction of rat heart. In male rats the BChE activity was 61.4 n moles/min/mg protein, while in the female rats the activity was 26 % higher. In diabetic males BChE activity

Table 4. Substrate kinetics of soluble form of cardiac BChE in the female rats.

Animals	Component I		Component II	
	Km	Vmax	Km	Vmax
Control (8)	0.25±0.032	23.3±1.32	2.20±0.13	53.8±1.93 [a]
Diabetic(8)	0.26±0.033	24.1±1.75 [c,#]	1.74±0.06 [b]	47.9±2.02 [c,#]
Insulin treated diabetic (9)	0.18±0.015	43.0±3.86	1.63±0.11	82.2±3.49

Results are expressed as mean±S.E.M. of number of independent observations indicated in the parentheses.

Units : Km = mM; Vmax = n mole / min / mg protein.

a, $p<0.01$, b, $p<0.005$ and c, $p<0.001$ compared with corresponding control.

#, $p<0.001$ compared with corresponding diabetic.

Table 5.   BChE activity in the membrane-bound fraction of rat heart.

Animals	Control	Diabetic	Insulin treated diabetic
Male	61.4±1.76(6)	135.6±5.66(8)^{b}	173.0±6.95(9)$^{b,\#}$
Female	77.1±3.01(6)	99.5±5.65(8)^{a}	128.5±9.22(11)$^{b,@}$

Results are expressed as mean±S.E.M. of number of independent observations indicated in the parentheses.

BChE activity = n mole / min / mg protein.

a, $p<0.005$ and b, $p<0.001$ compared with corresponding control.

@, $p<0.02$ and #, $p<0.001$ compared with corresponding diabetic.

increased significantly by 121 % as compared to the control. After insulin treatment there was a further significant increase of 28 % as compared to the diabetics; compared to the control males the activity was 182 % high. The same pattern was obtained for BChE activity in the female rats. However, the magnitude of increase was much less. Thus in the diabetic females the BChE activity increased only by 29 % as compared to the controls. After insulin treatment BChE activity increased further by 29 % as compared to the diabetic female rats and was 67 % higher as compared to the control female rats (Table 5).

Typical substrate saturation curves for BChE from membrane-bound fraction of male rat heart are shown in Figure 3. The corresponding typical Eadie-Hofstee plots (Figure 4) showed presence of two components of BChE in membrane-bound fraction from male rat heart. Data in Table 6 show Km and Vmax values derived from substrate kinetics of BChE from membrane-bound fraction of male rat heart. In control male rats the component I had a Km of 0.59 mM and Vmax of 39.4 n moles/min/mg protein, while component II had Km of 2.45 mM and Vmax 80.8 n moles/min/mg protein. In the diabetic male rats Km of component I decreased significantly by 59 % and the Vmax of the two components increased significantly by 74 % and 99 % respectively. After insulin treatment the Km of component I increased but it was still low compared to the male controls. Interestingly, Km of component II decreased

Figure 3.

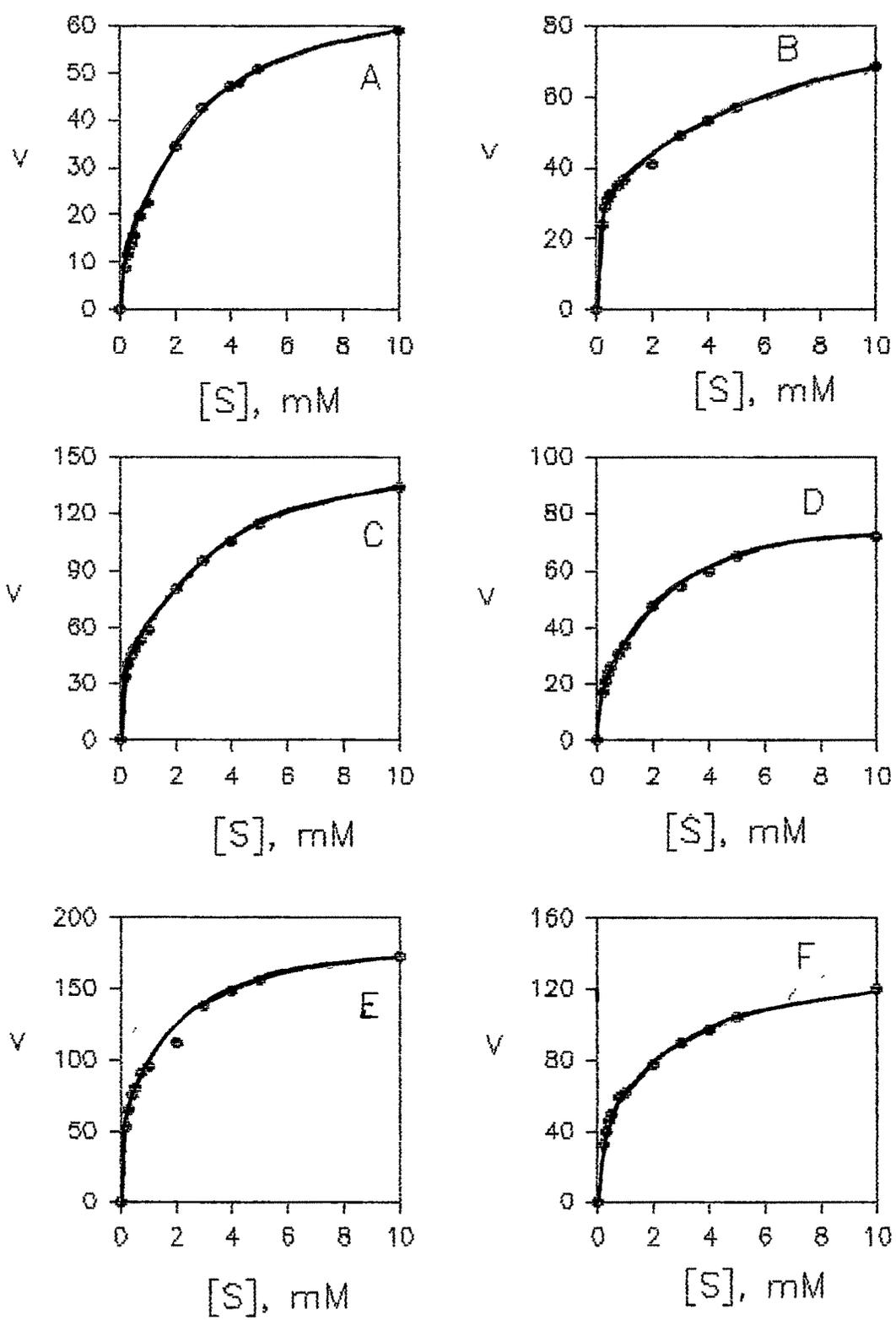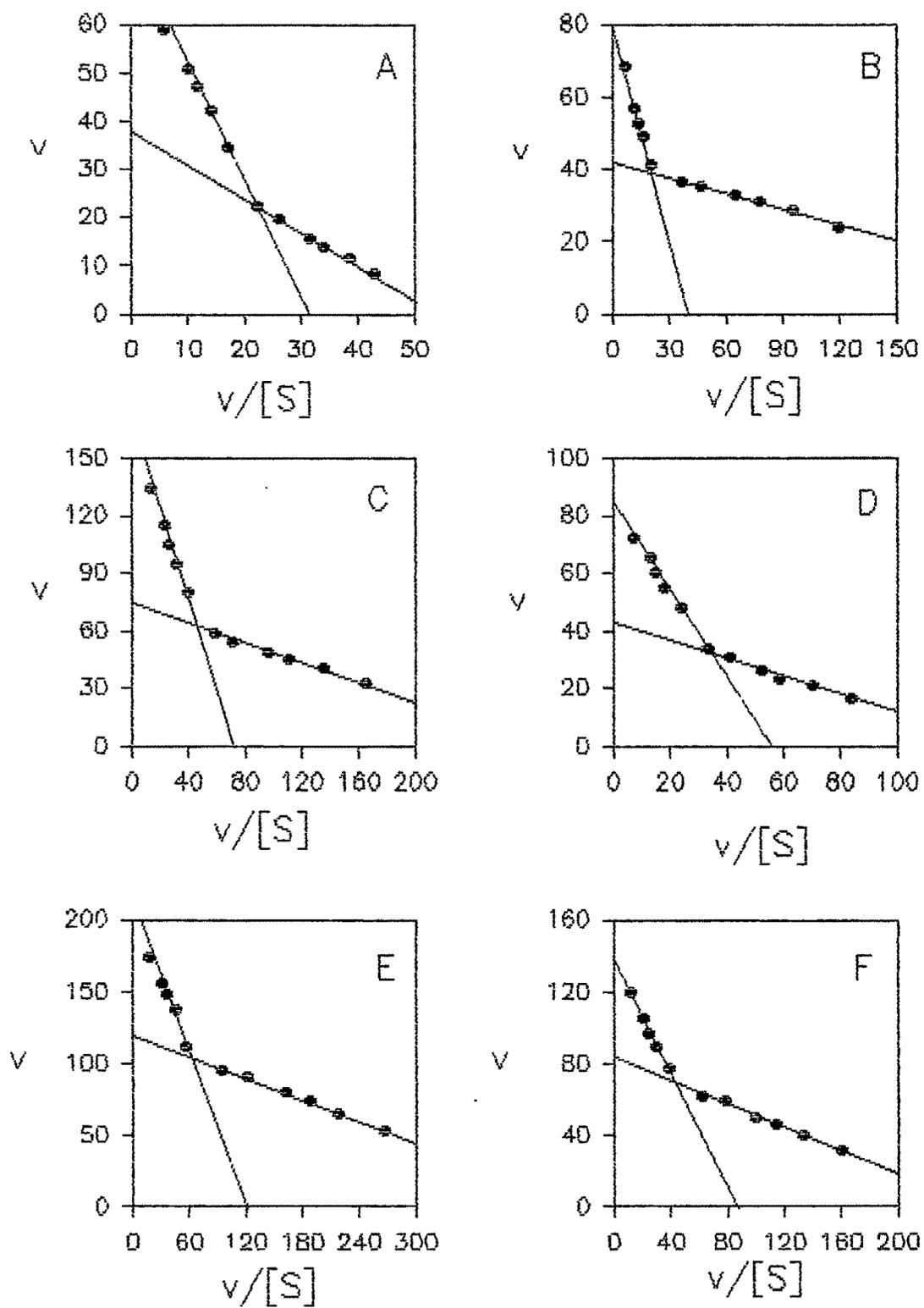Figure 4.

Table 6. Substrate kinetics of membrane-bound form of cardiac BChE from the male rats.

Animals	Component I		Component II	
	Km	Vmax	Km	Vmax
Control (6)	0.590±0.089	39.4±4.21	2.45±0.22	80.8± 4.06
	c	d		d
Diabetic (8)	0.239±0.030	68.8±3.08	2.12±0.08	160.8± 6.14
	a,@	d,$	b,@	d,#
Insulin treated diabetic (11)	0.352±0.040	120.1±4.86	1.61±0.21	205.5±10.59

Results are expressed as mean±S.E.M. of number of independent observations indicated in the parentheses.

Units : Km = mM; Vmax = n mole / min / mg protein.

a, p<0.05; b, p<0.02; c, p<0.005 and d, p<0.001 compared with corresponding control.

@, p<0.05; #, p<0.005 and $, p<0.001 compared with corresponding diabetic.

after insulin treatment but the Vmax of both the components increased further compared to the diabetic male rats.

Typical substrate saturation curves and the typical Eadie-Hofstee plots for BChE from membrane-bound fraction of female rat heart are shown in Figures 3 and 4 respectively. Data in Table 7 show the Km and Vmax values of the two components of BChE in membrane-bound fraction of female rat heart. As in the case of males, even in the females two components of BChE were present (Figure 4). Thus in the control group component I had low Km of 0.183 mM which was about 3 times lower compared to the control males. The Vmax was 39.7 n mole/min/mg protein, which compared well with the corresponding value of Vmax for the males. For component II the Km was 1.92 mM which was again significantly low compared to the corresponding value for the males. The Vmax for component II was comparable for both the sexes i.e. around 80 n mole/min/mg protein. In the diabetic state Km and Vmax of component I almost doubled while Vmax of component II increased by 29 %. After insulin treatment Km of component I decreased somewhat but was still higher than the control females. In the Km of component II there was a slight decrease. Vmax of component I further increased after insulin treatment significantly by 38 % as compared to the diabetic female rats and was 2.2 fold higher as compared to the control female rats. Similar pattern was obtained for Vmax of component II. However, the extent of increase was much less compared to the corresponding males (Table 6).

Table 7. Substrate kinetics of membrane-bound form of cardiac BChE form the female rats.

Animals	Component I		Component II	
	Km	Vmax	Km	Vmax
Control (6)	0.183 ± 0.014	39.7 ± 0.76	1.92 ± 0.07	83.8 ± 3.76
	a	d		b
Diabetic(8)	0.359 ± 0.028	63.5 ± 4.26	1.82 ± 0.06	108.2 ± 7.35
	b	d,@	c,#	d,@
Insulin treated diabetic (9)	0.276 ± 0.028	87.6 ± 7.77	1.52 ± 0.11	143.1 ± 10.14

Results are expressed as mean$\pm$S.E.M. of number of independent observations indicated in the parentheses.

Units : Km = mM; Vmax = n mole / min / mg protein.

a, $p < 0.05$; b, $p < 0.02$; c, $p < 0.01$ and d, $p < 0.001$ compared with corresponding control.

@, $p < 0.02$ and #, $p < 0.05$ compared with corresponding diabetic.

Discussion

From the data presented (Table 1) it is clear that in the control males, values for heart weight as % of body weight match well with the earlier reports of other researchers (17). In the diabetic rats there was a proportionate decrease in heart weight and insulin treatment brought about a proportionate restoration. Interestingly for the control females, the heart weight was relatively low compared to the males. However, unlike in the males, the tissue weight did not decrease in diabetic condition and remained higher even after insulin treatment. The results thus point out that basic difference in cardiac physiology existed in the males and females and that the response to diabetic state was also differential.

The difference became apparent even with respect to basal level of soluble form of BChE which was very high in the control females (Table 2). As compared to the males the value was 3.3 fold higher in the females. Similar pattern was noted even for the membrane-bound enzyme (Table 5), however the BChE activity in the females was only about 25 % higher as compared to the control males. The diabetic state had differential effect on the soluble form of BChE in that in the males the activity increased by 3 fold but in the females there was a small but reproducible (20 %) decrease. Insulin

treatment resulted in further substantial increase in the activity in both males and females. This would indicate that the trend of increased BChE activity in diabetic rats was not restored by insulin treatment. This is consistent with the earlier observations that all the maladies of diabetes are not corrected by insulin treatment (23).

For the membrane-bound form of BChE, diabetic state resulted in substantial increase in the activity in both males and females (2.2 and 1.3 fold respectively). Obviously the magnitude of increase was much lower in the females. As in the case of the soluble BChE, the increased activity in diabetes was not reversed by insulin treatment. Even in the insulin treated females the BChE activity was low by about 27 % as compared to the males.

The heart has adrenergic as well as cholinergic innervations (24). The increased BChE activity in the membrane-bound fraction in diabetics may be a compensatory mechanism towards efficient functioning of the cholinergic system. The activity in the soluble fraction may be a reflection of the pattern noted for the membrane-bound form. It is also noteworthy that the increase in the membrane-bound form of BChE in the females was of lesser magnitude than in the males. This may imply that the compensatory mechanism was working much less efficiently in the females.

One could view these data in terms of the ratios of specific

activity of soluble and membrane-bound forms. The ratio of soluble to membrane-bound form of the enzyme was 0.26 in control males but was much high i.e. 0.68 in the control females. In the diabetic males the ratio increased but only slight eg. (0.33) and the trend of increased ratios continued (0.44) in the insulin treated diabetic males. In the females under all the conditions the ratio was always higher than in the corresponding males (0.68, 0.42 and 0.57 for control, diabetic and insulin treated diabetic groups respectively). It may hence be suggested that there is basic biochemical differences in the membrane properties of the female heart especially with respect to the binding of BChE; the extent of membrane binding of BChE was much less in the female heart.

The differences in basic cardiac physiology became more elaborate from the substrate kinetics studies (Table 3, 4, 6 and 7) and from temperature kinetic studies (Chapter 8 of the thesis). Thus diabetes statues resulted in a generalized decrease in the Km of the two components of soluble BChE, which continued even after insulin treatment in both males and females. Decreased Km is consistent with the assumption (vide supra) that increased Vmax may be a compensatory mechanism.

In the membrane-bound form of the enzyme in the males the pattern was parallel to that seen for the soluble form. However, interestingly in the females Km of component I (high

affinity component) increased in diabetic and insulin-treated diabetic rats, which was opposite to that seen in the males; Km of component II was not affected.

Although BChE makes up about 85 % of the total cardiac cholinesterase activity (7), the physiological hydrolysis of acetylcholine is believed to be effected by AChE (7). If the presient resulted are any pointer, it may be presumed that a similar pattern could be discerned for the AChE activity.

Hence the results of the present study suggest that primarily the lesion in the diabetic female heart may be differential and more severe than in the diabetic males. Besides, the compensatory mechanism may also be functioning less efficiently in the females. Thirdly, in the female heart the membrane alterations are such that it lowers the affinity of the enzyme for the substrate, which may further add to the cardiac complications. The membrane alterations can thus severely affect the cardiac functioning.

Possibly because of these reasons the diabetic females may be more succeptible to coronary heart disease (CHD) and congestive heart failure (CHF) (3).

Summary

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 male rats the heart weight was relatively high as compared to females. In the males the heart weight decreased in diabetic condition and increased after higher after insulin treatment, while in the females no effect on heart weight was observed.

In control female rats the soluble BChE activity in the heart was very high compared to the males. Similar pattern was observed also for the membrane-bound BChE.

The soluble BChE activity increased in male but decreased in female diabetic rats, insulin treatment resulted in substantial increase in the activity in both males and females.

The membrane bound BChE activity increased in diabetic state in males as well as in the female rats.

Diabetes state resulted in a generalized decrease in the Km of the two components of soluble BChE, which continued even after insulin treatment in both males and females.

In the membrane-bound form of the enzyme, in the males the pattern was parallel to that seen for the soluble form. In the females for the membrane-bound form of BChE, Km of component I increased in diabetic and insulin-treated rats, while Km of component II was not affected.

Figure legends

Figure 1 Typical substrate saturation curves for rat heart soluble form of BChE. (A) Control male (B) Control female (C) Diabetic male (D) Diabetic female (E) Insulin treated diabetic male (F) Insulin treated diabetic female rats. Other experimental details are as described in the text.
Enzyme activity v in n mole/min/mg protein

Figure 2 Typical Eadie-Hofstee plots for rat heart soluble form of BChE. (A) Control male (B) Control female (C) Diabetic male (D) Diabetic female (E) Insulin treated diabetic male (F) Insulin treated diabetic female rats. Other experimental details are as described in the text and in legend to Figure 1.

Figure 3 Typical substrate saturation curves for rat heart membrane-bound fraction of BChE. (A) Control male (B) Control female (C) Diabetic male (D) Diabetic female (E) Insulin treated diabetic male (F) Insulin treated diabetic female rats. Other experimental details are as described in the text.
Enzyme activity v in n mole/min/mg protein.

Figure 4 Typical Eadie-Hofstee plots for rat heart membrane-bound fraction of BChE. (A) Control male (B) Control female (C) Diabetic male (D) Diabetic female (E) Insulin treated diabetic male (F) Insulin treated diabetic female rats. Other experimental details are as described in the text and in legend to figure 3.

References

1) Dorman J. S. (1997) Molecular epidemiology of insulin-dependent diabetes mellitus. Epidemiologic Rev. 19, 91-98.

2) Diabetes complication. In : Moy C. S., Ed. Diabetes 1993 Vital statistics. The American Diabetes Association, 1993.

3) Pyorala K. (1985) Diabetes and coronary heart disease, Acta Endocrinol. 110, 11-19.

4) Stamler J. (1987) Epidemiology, established risk factors and the primary prevention of coronary heart disease. In cardiology. Parmley W. W. and Chatterjee K. (Eds.) Philadelphia, PA, Lippincott. pp. 1-41.

5) Pyorala K. Lakso M. and Unsitupa M. (1987) Diabetes and atherosclerosis : epidemiological view. Diabetes Metab. Rev. 3, 463-524.

6) Seager M. J., Singhal P. K., Orchard R., Pierce G. N. and Dhalla N. S. (1984) Cardiac cell damage : A primary myocardial disease in streptozotocin-induced chronic diabetes. Br. J. Exptl. Pathol. 65, 613-623.

7) Chatonnet A. and Lockridge O. (1989) Comparisson of butyrylcholinesterase and acetylcholinesterase. Biochem. J., 260, 625-634.

8) Patel B. N., Macknes M. I., Harty D. W., Arrol S., Boot-Handforf R. P. and Durrington P. N. (1990) Serum esterase activities and hyperlipidaemia in the streptozotocin-diabetic rat. Biochim. Biophys. Acta., 1035, 113-116.

9) Oreskovic K. and kunec-Vajic E. (1992) Pseudocholinesterase in alloxan-diabetic rats. Res. Commun.

Clin. Pathol. Pharmacol. 78, 117-120.

10) Lockridge O., Eckerson H. W. and La Du B. N. (1979) Interchain disulfide bonds and subunit organization in human serum cholinesterase. J. Biol. Chem. 254, 8324-8330.

11) Masson P. (1979) Formes molecularies multiple de la butyrylcholinesterase du plasma humain I. Parameters molecularies apparents et ebauche de la structure quaternaire., Biochim. Biophys. Acta. 578, 493-504.

12) Masson P. (1989) A naturally occuring molecular form of human plasma cholinesterase is an albumin conjugate. Biochim. Biophys. Acta, 988, 258-266.

13) Masson P. (1991 a) Structure and functional investigations of cholinesterase by means of affinity electrophoresis. Cell. Molec. Neurobiol. 11, 173-190.

14) Masson P. (1991 b) Molecular heterogeneity of human plasma cholinesterase. In : Cholinesterases : Structure, Function, Mechanism, Genetics and cell Biology, Eds. J. Massoulie, F. Bacou, E. A. Barnard, A. Chatonnet, B. P. Doctor and D. M. Quinn., American. Chemical Society, Washington D. C. pp. 42-46.

15) Lyles J. M., Silman I., DiGiamberadino L., Courand J. Y. and Barnard E. (1982) Compression of the molecular forms of the cholinesterase in tissues of normal and dystrophic chickens. J. Neurochem. 38, 1007-1021.

16) Bahmak B. R. and Gold A. H. (1982) Effect of alloxan diabetes on the turnover of rat liver glycogen synthase. J. Biol. Chem. 257, 8775-8780.

17) Nerurkar M. A., Satav J. G. and Katyare S. S. (1988)

Insulin-dependent changes in lysosomal cathepsin-D activity in rat liver, kidney, brain and heart. **31**, 19-122.

18) Bisso Q. M., Briancesco R. and Michalek H. (1991) Size and charge isomers of acetylcholinesterase in the cerebral cortex of young and aged rats. Neurochem Res. **16**, 571-575.

19) Khandkar M. A., Mukherjee E., Parmar D. V. and Katyare S. S. (1995) Alloxan-diabetes alters kinetic properties of the membrane-bound form, but not of the soluble form, of acetylcholinesterase in rat brain. Biochem. J. **307**, 647-649.

20) Ellman G. L., Courtney K. V., Anders V. and Featherstone R. M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. **7**, 88-95.

21) Dixon M. and Webb C. (1979) Enzymes, Longman, London, pp. 47-206.

22) Ku D. D. and Sellers B. M. (1982), Effects of streptozotocin diabetes and insulin treatment on myocardial sodium pump and contractility of the rat heart. J. Pharmacol. Exptl. Therapeut. **222**, 395- 400.

23) Ido Y., Vindigni A., Change K., Stram L., Chance R., Heath W. F., DiMarchi R. D., DiCera E. and Williamson J. R. (1997) Prevention of vascular and neural dysfunction in diabetic rats by C-peptide. Science, **277**, 563-566.

24) Introduction to the cardiovascular system. Chapter 5 (1996) In : Book and Taylor's Physiological Basis of Medical Practice, West J. B. Ed., Twelfth Edition, B. I. Waverly Pvt. Ltd., New Delhi.