

Effect of alloxan-induced diabetes on serum and cardiac butyrylcholinesterases in the rat

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Abstract

Elevated serum butyrylcholinesterase (BChE) activity in the diabetic rat, mouse and human is very evident. The source of the increased level of BChE in the diabetic condition is not known. The effect of diabetes on cardiac BChE has not been studied so far, in spite of high BChE levels in the heart. In the present study, we investigated the effect of alloxan-induced diabetes on serum and on the soluble as well as the membrane-bound form of cardiac BChE activity and their substrate kinetics. We included rats of both sexes in the study. Serum BChE activity increased only in male diabetic rats (2.3-fold), while the activities of the soluble as well as the membrane-bound form of cardiac BChE activity increased 2.2- to 2.8-fold in male diabetic rats. A smaller increase (30%) was observed

in the activity of the membrane-bound form of cardiac BChE in female diabetic rats. A slight reduction in BChE activity was observed in male and female rats after insulin treatment. The activity ratio of the soluble to the membrane-bound form of cardiac BChE was higher in diabetic and insulin-treated diabetic rats as compared with controls. The K_m values of component II of the serum and soluble forms of cardiac BChE were comparable. In conclusion, the diabetes-induced increase in serum and cardiac BChE activity was sex dependent. Insulin was not able to rectify the diabetes-induced abnormalities in serum and cardiac BChE activity. The heart could be one of the possible sources of the increased level of serum BChE.

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Introduction

Vertebrates possess two cholinesterases corresponding to two distinct genes: acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8). The two enzymes are distinguished primarily on the basis of their substrate specificity (Massoulié *et al.* 1993). AChE is thought to participate in the hydrolysis of acetylcholine, terminating its physiological action (Chatonnet & Lockridge 1989, Massoulié *et al.* 1993). The function of BChE remains a puzzle. It has been shown that BChE is involved in the degradation of succinylcholine (a myorelaxant used in surgical operations), hydrolysis of drugs such as heroin, detoxification of plant esters and phytotoxins ingested in the diet, regulation of plasma choline levels, and in lipid/lipoprotein metabolism (Funnell & Oliver 1965, Kutty 1980, Lockridge *et al.* 1980, Valentino *et al.* 1981, Shirai *et al.* 1988, 1991).

A rise in serum BChE activity in the diabetic rat, mouse and human (22%–270%) has been reported (Kutty *et al.* 1981, Patel *et al.* 1990, Annapurna *et al.* 1991, Oreskovic & Kunec-Vajic 1992, Abbott *et al.* 1993). BChE activity was also found to be increased significantly in the plasma, pancreas and adipose tissue (33–100%) of alloxan-induced diabetic rats (Oreskovic & Kunec-Vajic 1992). BChE

activity was found to be higher in all tissues in control female rats when compared with control male rats. Oreskovic & Kunec-Vajic (1992) also observed similar sex differences in BChE activity under diabetic conditions. It has been shown that the rise in BChE activity in plasma is not related to the level of BChE production in the liver. It has been speculated that adipose tissue might be a site of synthesis of increased levels of serum BChE (Kutty 1980).

The increase in serum BChE activity might be a response to hypertriglyceridemia or it might reflect a decreased removal from the circulation as a consequence of diabetic renal microangiopathy (Patel *et al.* 1990). A positive correlation was found between serum BChE activity and serum triacylglycerol concentrations in hypertensive patients, obese patients and patients with hypercholesterolemia and hyperlipoproteinemia (Cucuianu *et al.* 1975, Chu *et al.* 1978, Magarian & Dietz 1987). The injection of heparin appears to activate lipoprotein lipase in diabetic rats, shown by a marked fall in serum triglyceride and total low density lipoprotein levels but not in BChE activity (Annapurna *et al.* 1991). A relationship between serum lipid/lipoprotein metabolism and BChE activity in diabetes lacks any strong supportive evidence.

This literature survey thus reveals that a rise in serum BChE activity in the diabetic condition is very evident,

but the source of the increased levels of BChE is still unknown. Surprisingly, the effect of diabetes on cardiac BChE has not been studied as yet, even though the heart in higher vertebrates is one of the tissues containing a large amount of BChE. In the adult hearts of higher vertebrates, 85% of total cholinesterase is BChE and only 15% is AChE (Chatonnet & Lockridge 1989).

In view of the above, the main objective of the present study was to investigate the effect of alloxan-induced diabetic and insulin-treated alloxan-induced diabetic conditions on serum and cardiac BChE activity. For easy comparison between different isoforms of BChE in serum and heart, substrate kinetics were also studied. Because of reports showing a sex-dependent effect of diabetes on BChE activity, we included rats of both sexes in the present study.

Materials and Methods

Chemicals

Butyrylthiocholine iodide (BCTI) and Triton X 100 were purchased from Sigma Chemical Co., St Louis, MO, USA. 5,5'-Dithio-bis (2-nitrobenzoic acid) (DTNB) was purchased from SRL (Mumbai, India) and NPH insulin from Knoll Pharmaceuticals Ltd (Ahmedabad, India). All other chemicals were of analytical reagent grade and were purchased locally.

Animals

Male and female albino rats of the Charles Foster strain weighing between 180 and 220 g were used. After fasting for 24 h the animals were given a single s.c. injection of freshly prepared alloxan solution using saline (0.9% (w/v) NaCl) as vehicle, at a dose of 12 mg alloxan/100 g body weight (Bahnak & Gold 1982). The control animals received saline vehicle only. The diabetic state was ascertained in terms of loss of body weight, polyuria, glycosuria, polydipsia, polyphagia and blood glucose levels (Nerurkar *et al.* 1988). Symptoms of diabetes were observed within a week of alloxan injection. One group of animals was used as a diabetic group (no treatment). Another group of diabetic animals received NPH insulin s.c. at a dose of 8 U/kg body weight twice daily for 7 days consecutively starting at the end of 22 days of alloxan treatment (Nerurkar *et al.* 1988); the last dose of insulin was given 12 h prior to killing the animals. This group was used as the insulin-treated diabetic group. At the end of 1 month after treatment with alloxan or vehicle, animals were killed. The protocol was approved by the Departmental Animal Ethics Committee.

Separation of serum

The blood was collected and allowed to clot at room temperature and serum was collected after centrifugation

at 475 g for 8 min in a clinical centrifuge. The straw-colored supernatant was carefully decanted.

Isolation of soluble and membrane-bound forms of BChE from the heart

Isolation of soluble and membrane-bound forms of BChE from the heart was achieved essentially by following the procedure of Bisso *et al.* (1991) as described earlier (Dave *et al.* 2000, 2002). Briefly, the tissue (heart) from male and female rats of all groups was quickly removed after decapitation and placed in beakers containing chilled (0–4 °C) 38 mM Tris–HCl buffer at pH 8.5. The tissues were repeatedly washed with the same buffer to remove blood and 10% (w/v) homogenates were prepared using a Potter–Elvehjem type glass–teflon homogenizer. The supernatant obtained after centrifugation at 100 000 g for 1 h was used as the source of the soluble form of the enzyme. The pellet was extracted in the same volume of 38 mM Tris–HCl buffer, pH 8.5, containing 0.25% Triton X-100 and after centrifugation at 100 000 g for 1 h the supernatant was used as the source of the membrane-bound enzyme. All the operations were carried out at 0–4 °C.

Assay of BChE activity

The enzyme activities were determined according to the method of Ellman *et al.* (1961), with some modifications (Dave *et al.* 2000, 2002). The assay mixture contained in a volume of 1.0 ml was 50 mM Tris–HCl buffer, pH 8.0, containing 0.38 mM DTNB; 10–50 µg protein served as a source of tissue enzymes, while 5–10 µl serum was used as the source of the enzyme for serum. The linear rate of reaction recorded over a period of 60–90 s at 37 °C was used for calculation of the rate of the reaction. The DTNB solution was prepared by dissolving 10 mg DTNB + 5 mg NaHCO₃ in 10 ml Tris–HCl buffer (Dave *et al.* 2000, 2002).

For substrate kinetic studies, the concentration of BCTI ranged from 0.05 to 10 mM in serum and 0.2 to 10 mM in the soluble and membrane-bound forms of the heart. For substrate kinetics, the data were analyzed by the Lineweaver–Burk, Eadie–Hofstee and Eisenthal Cornish–Bowden methods for the determination of apparent K_m and apparent V_{max} (Dixon & Webb 1979). Due to limitations in these methods of analysis, the K_m and V_{max} values presented in the present study are considered to be apparent. The values of apparent K_m and apparent V_{max} obtained by the three methods of analysis were in close agreement and were averaged. The values are given as means ± S.E.M. Analyses of substrate kinetics data were carried out by employing Sigma Plot, Version 5.0 (Dave *et al.* 2000, 2002).

Protein was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Table 1 Physiological parameters in alloxan-treated rats. Results are expressed as means \pm S.E.M. of the number of independent observations indicated in parentheses

Parameter	Sex	Control (16)	Diabetic (16)	Insulin-treated diabetic (13)
Body weight (g)	Male	248 \pm 9	183 \pm 6**	245 \pm 6 ^b
	Female	243 \pm 7	174 \pm 6**	221 \pm 10 ^b
Body sugar (mM)	Male	5.9 \pm 1.6	24 \pm 2**	4.8 \pm 0.3 ^b
	Female	6.7 \pm 0.5	37 \pm 4.4**	8.1 \pm 0.3 ^b
Urine volume/day (ml)	Male	2.5 \pm 0.2	43 \pm 5.4**	2.2 \pm 0.4 ^b
	Female	2.9 \pm 0.4	47.0 \pm 4.1**	3.1 \pm 0.5 ^b
Sugar (mg/ml urine)	Male	N.D.	26 \pm 1.6	N.D.
	Female	N.D.	15 \pm 0.6	N.D.
Sugar excretion (g/day)	Male	—	1.1 \pm 0.1	—
	Female	—	0.76 \pm 0.07	—
Heart weight (g)	Male	0.67 \pm 0.08	0.52 \pm 0.02	0.68 \pm 0.02 ^b
	Female	0.45 \pm 0.02	0.46 \pm 0.04	0.66 \pm 0.02** ^b
Heart weight (% of body weight)	Male	0.28 \pm 0.01	0.29 \pm 0.01	0.28 \pm 0.01
	Female	0.21 \pm 0.01	0.27 \pm 0.01**	0.30 \pm 0.02**

N.D., not detectable.

** $P < 0.005$ compared with corresponding control; ^b $P < 0.005$ compared with corresponding diabetic.**Table 2** Effect of alloxan-induced diabetes on serum BChE activity (nmol/min per ml serum) in male and female rats. BCTI (4 mM) was used as substrate in the assay. Results are expressed as means \pm S.E.M. of the number of independent observations indicated in parentheses

Sex	Control	Diabetic	Insulin-treated diabetic
Male	215 \pm 10 (9)	497 \pm 45 (10)**	394 \pm 31 (7)**
Female	941 \pm 95 (9)	958 \pm 83 (10)	888 \pm 90 (7)

** $P < 0.005$ compared with corresponding control.

Protein yield was comparable in soluble and membrane-bound forms of the heart in all experimental and control groups. Statistical evaluation of the data was performed using the Student's *t*-test.

Results

Effect of alloxan-induced diabetes on physiological parameters

Polyuria, glycosuria and hyperglycemia were observed in diabetic male and female rats (Table 1). Insulin treatment of diabetic animals was able to control these characteristics of diabetes in both sexes.

Elevated serum BChE activity in diabetic males but not in females

Our aim was to study serum BChE activity in diabetic and insulin-treated diabetic male and female rats. The serum BChE activity was higher by 4.4-fold in the control female rats when compared with the control male rats (Table 2).

In the diabetic male rats, the serum BChE activity was higher by 2.3-fold ($P < 0.005$) and remained elevated (1.8-fold, $P < 0.005$) after insulin treatment when compared with the control group. A statistically non-significant decreasing trend (21%) in serum BChE activity was observed in insulin-treated diabetic male rats when compared with the diabetic male group. By contrast, in females no statistically significant differences were found in BChE activity among three groups, i.e. control, diabetic and insulin-treated diabetic.

In brief, the BChE activity in control female rats was higher than in the control males. The serum BChE activity was higher in diabetic and insulin-treated diabetic male rats. Diabetes or insulin treatment had no effect on serum BChE activity in the female rats.

Effect of alloxan-induced diabetes on substrate kinetics of serum BChE

In view of the dramatic changes in serum BChE activity, we extended our studies and looked at the substrate kinetic

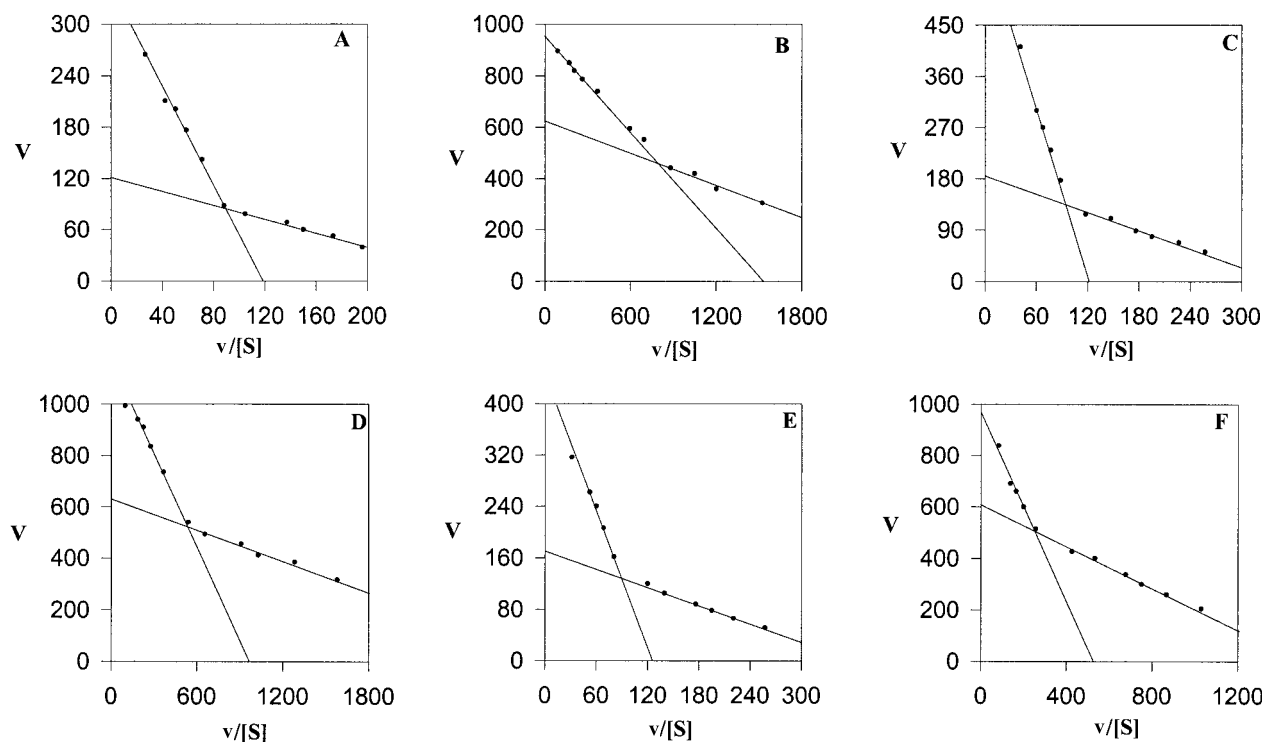


Figure 1 Typical Eadie–Hofstee plots for the rat serum BChE for (A) control male, (B) control female, (C) diabetic male, (D) diabetic female, (E) insulin-treated diabetic male and (F) insulin-treated diabetic female rats. Experimental details are given in the text. For determination of substrate kinetics of BChE, BCTI was used as the substrate over a concentration range of 0.05–10 mM. The ordinate represents the reaction velocity (v), while the abscissa represents the $v/[S]$ ratios. Reaction velocity = nmol/min per mg protein. $v/[S]$ = reaction velocity divided by the corresponding substrate concentration.

properties of serum BChE under different experimental conditions. Typical Eadie–Hofstee plots for serum BChE for male and female rats are shown in Fig. 1 from which it can be inferred that two components of BChE are present in the serum of male and female rats from all three experimental groups. The presence of two components of BChE was confirmed by plotting Eadie–Hofstee plots for the data following the Michaelis–Menten kinetic equation for two enzymes acting on the same substrate: $v = (V_{\max 1} \times [S]) / (K_m 1 + [S]) + \{V_{\max 2} \times [S] / (K_m 2 + [S])\}$. Data in Table 3 show K_m and V_{\max} values derived from substrate kinetic analysis for serum BChE from the male and female rats.

The K_m of component II was higher in male (65%, $P < 0.05$) and female (53%, $P < 0.05$) diabetic rats when compared with the corresponding control groups. The insulin treatment was able to rectify this defect in animals of both sexes. In diabetic male rats, the V_{\max} of components I and II was higher by 63% ($P < 0.05$) and 144% ($P < 0.005$) respectively when compared with the male controls. In the males after insulin treatment, the V_{\max} of component II remained higher by 65% ($P < 0.005$) when compared with the control males. However, the V_{\max} of

component II decreased by 27% ($P < 0.05$) as compared with the male diabetics, but was still about 80% ($P < 0.005$) higher than in the control males. No statistical differences were observed in the V_{\max} of components I and II in all three female experimental groups.

In brief, the V_{\max} of both components increased in male diabetic rats and remained elevated after insulin treatment, while in the females the V_{\max} of both the components were comparable among all three experimental groups; i.e. the diabetic state or insulin treatments had no effect.

Effect of alloxan-induced diabetes on heart weight

It was of interest to note the weight of the heart in all the experimental groups. A statistically non-significant decreasing trend (23%) was observed in heart weight in diabetic males (Table 1). In insulin-treated diabetic males, the heart weight increased by 30% ($P < 0.005$) and was comparable with the control males. The heart weight when expressed as percent of body weight was comparable among all three experimental groups. In control female rats, the heart weight was lower by 33% as compared with the control male rats (Table 1). The heart weights were

Table 3 Effect of alloxan-induced diabetes on substrate kinetics (K_m , mM; V_{max} , nmol/min per ml serum) of serum BChE in male and female rats. Results are expressed as means \pm S.E.M. of the number of independent observations indicated in parentheses

Sex	Group	Component I		Component II	
		K_m	V_{max}	K_m	V_{max}
Male	Control (9)	0.49 \pm 0.06	146 \pm 14	2.2 \pm 0.19	264 \pm 13
	Diabetic (10)	0.51 \pm 0.08	237 \pm 35*	3.6 \pm 0.48*	644 \pm 57**
	Insulin-treated diabetic (7)	0.31 \pm 0.07	240 \pm 23**	2.3 \pm 0.36	471 \pm 39***
Female	Control (8)	0.34 \pm 0.05	708 \pm 81	1.1 \pm 0.12	1049 \pm 107
	Diabetic (10)	0.32 \pm 0.05	601 \pm 105	1.7 \pm 0.24*	1020 \pm 142
	Insulin-treated diabetic (6)	0.27 \pm 0.03	698 \pm 56	1.3 \pm 0.14	1062 \pm 65

* $P < 0.05$ and ** $P < 0.005$ compared with corresponding control value; ^a $P < 0.05$ compared with corresponding diabetic value.

Table 4 BChE activity (nmol/min per mg protein) in soluble and membrane-bound fractions of rat heart. BCTI (4 mM) was used as substrate in the assay. Results are expressed as means \pm S.E.M. of the number of independent observations indicated in parentheses

Fraction of heart	Sex	Control	Diabetic	Insulin-treated diabetic
Soluble	Male	16 \pm 1 (8)	45 \pm 2 (8)**	76 \pm 2 (11)** ^b
	Female	52 \pm 2 (8)	42 \pm 2 (8)*	74 \pm 3 (9)** ^b
Membrane-bound	Male	61 \pm 2 (6)	136 \pm 6 (8)**	173 \pm 7 (9)** ^b
	Female	77 \pm 3 (6)	100 \pm 6 (8)**	129 \pm 9 (11)** ^a

* $P < 0.05$ and ** $P < 0.005$ compared with corresponding control; ^a $P < 0.05$ and ^b $P < 0.005$ compared with corresponding diabetic group.

comparable in control and diabetic females. In females, after insulin treatment the heart weight increased by 46% ($P < 0.005$) and 42% ($P < 0.005$) as compared with the control and diabetic females respectively. In diabetic females, the heart weight, when expressed as percent of body weight, was higher by 29% ($P < 0.005$) as compared with the control females. The heart weight, when expressed as percent of body weight, further increased (10%) after insulin treatment as compared with the diabetic female rats although this increase was not statistically significant. Compared with the control females the increase was 42% ($P < 0.005$) in the insulin-treated diabetic females.

In brief, the control female hearts were smaller than the hearts of control males. The values of heart weight and heart weight as percent of body weight revealed an increased size of the heart in diabetic and insulin-treated diabetic females when compared with the control females; this was not true for the males.

Effect of alloxan-induced diabetes on BChE activities in soluble and membrane-bound fractions of rat heart

In both males and females, the major portion of the enzyme (in terms of enzyme activity) was present in the

membrane-bound fraction as compared with the soluble fraction (Table 4). In the control females, the enzyme activities were higher by 230% and 26% in the soluble and membrane-bound fractions respectively, as compared with the control males. In the diabetic male rats, BChE activity in the soluble fraction was higher by 186% ($P < 0.005$) when compared with the controls. After insulin treatment of the diabetic male rats, the BChE activity in the soluble fraction further increased and remained elevated, while in the diabetic females the BChE activity in the soluble fraction was lower by 20% ($P < 0.05$) when compared with the female controls. However, after insulin treatment BChE activity increased by 41% ($P < 0.005$) and 77% ($P < 0.005$) as compared with the control and diabetic females respectively.

In male rats, an almost similar effect was observed in the membrane-bound fraction of BChE under diabetic and insulin-treated diabetic conditions as in the case of the soluble fraction. A similar pattern was obtained for BChE activity in the membrane-bound fraction in the female rats. However, the magnitude of increase in the BChE activity in the membrane-bound fraction was lower in the females when compared with the males.

Except in the case of the soluble fraction of cardiac BChE from diabetic female rats, in general, cardiac BChE

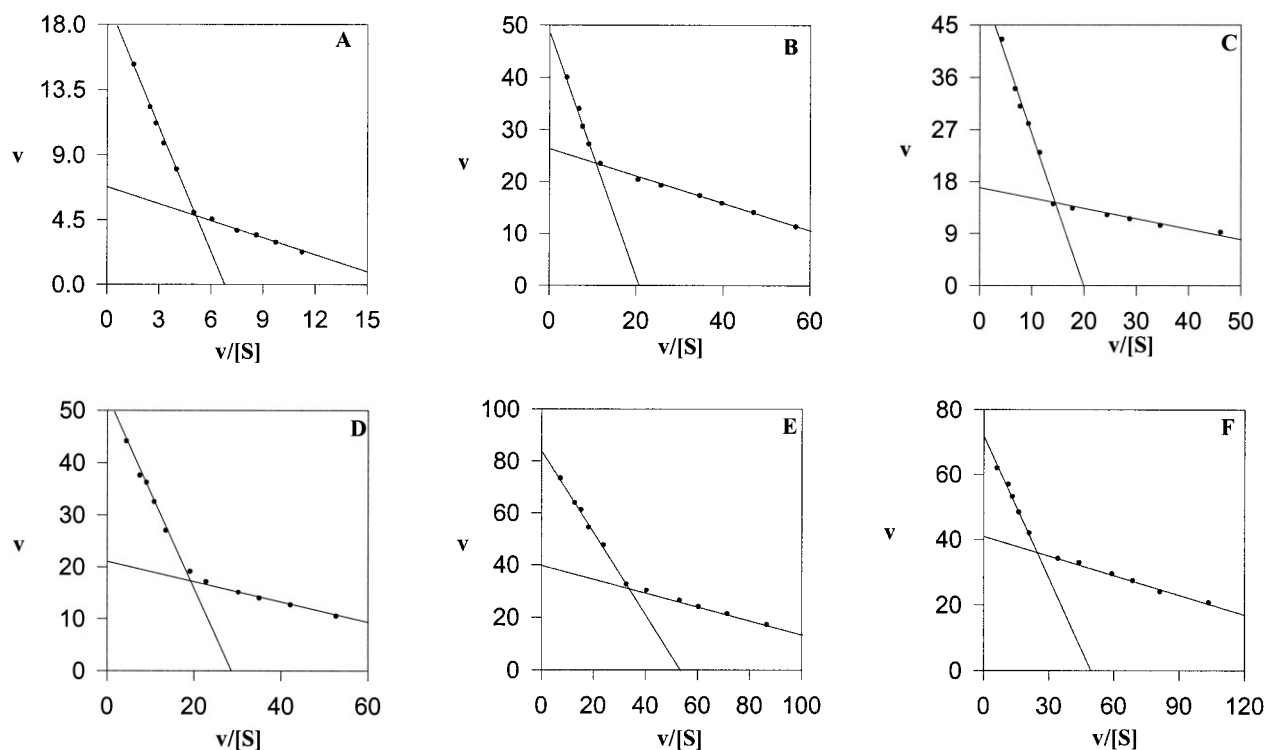


Figure 2 Typical Eadie–Hofstee plots for the rat heart soluble form of BChE for (A) control male, (B) control female, (C) diabetic male, (D) diabetic female, (E) insulin-treated diabetic male and (F) insulin-treated diabetic female rats. Experimental details are given in the text. For determination of substrate kinetics of BChE, BCTI was used as the substrate over a concentration range of 0.2–10 mM. The ordinate represents the reaction velocity (v), while the abscissa represents the $v/[S]$ ratios. Reaction velocity = nmol/min per mg protein. $v/[S]$ = reaction velocity divided by the corresponding substrate concentration.

activity was found to be elevated in the diabetic condition, and it was further increased in the insulin-treated rats. The magnitude of the increases was higher in the males as compared with the female rats.

Effect of alloxan-induced diabetes on the substrate kinetics of BChE from soluble and membrane-bound fraction of rat heart

In view of the drastic increase in cardiac BChE activity, it was of interest to study the effect of the alloxan-induced and insulin-treated alloxan-induced diabetic conditions on substrate kinetic properties of the soluble and membrane-bound fractions of cardiac BChE. Eadie–Hofstee plots for substrate kinetics of BChE from the soluble fraction of male and female rat heart are given in Fig. 2, while Fig. 3 shows typical Eadie–Hofstee plots for substrate kinetics of the membrane-bound fraction of cardiac BChE in the male and female rats. The pattern shows the presence of two components of BChE in both fractions (Figs 2 and 3). This was the same in the case of serum enzyme, where the presence of two components of BChE was confirmed by plotting Eadie–Hofstee plots for the data using the Michaelis–Menten kinetic equation for two enzymes act-

ing on the same substrate. Data in Table 5 give the K_m and V_{max} values derived from the substrate kinetics analysis of BChE in both fractions.

In diabetic males, the K_m of component I of BChE from the soluble fraction of the heart decreased by 44% ($P < 0.005$) as compared with the control males, while the K_m of component II was comparable among the diabetic and control male groups. The V_{max} of both components I and II increased by 173% ($P < 0.005$) and 211% ($P < 0.005$) respectively when compared with the control males. After insulin treatment, the K_m of component I was still lower (39%, $P < 0.005$) as compared with the control values, and the K_m of component II also decreased by 35% as compared with both the control ($P < 0.005$) and diabetic ($P < 0.005$) males. Insulin treatment also resulted in a statistically significant further increase in the V_{max} of both components.

In the diabetic condition, only the K_m of component II of BChE from the soluble fraction of the heart decreased by 21% ($P < 0.005$) when compared with the control females; even after insulin treatment the K_m of component II was the same as in the diabetic group. In females, the diabetic condition had no effect on the V_{max} of both

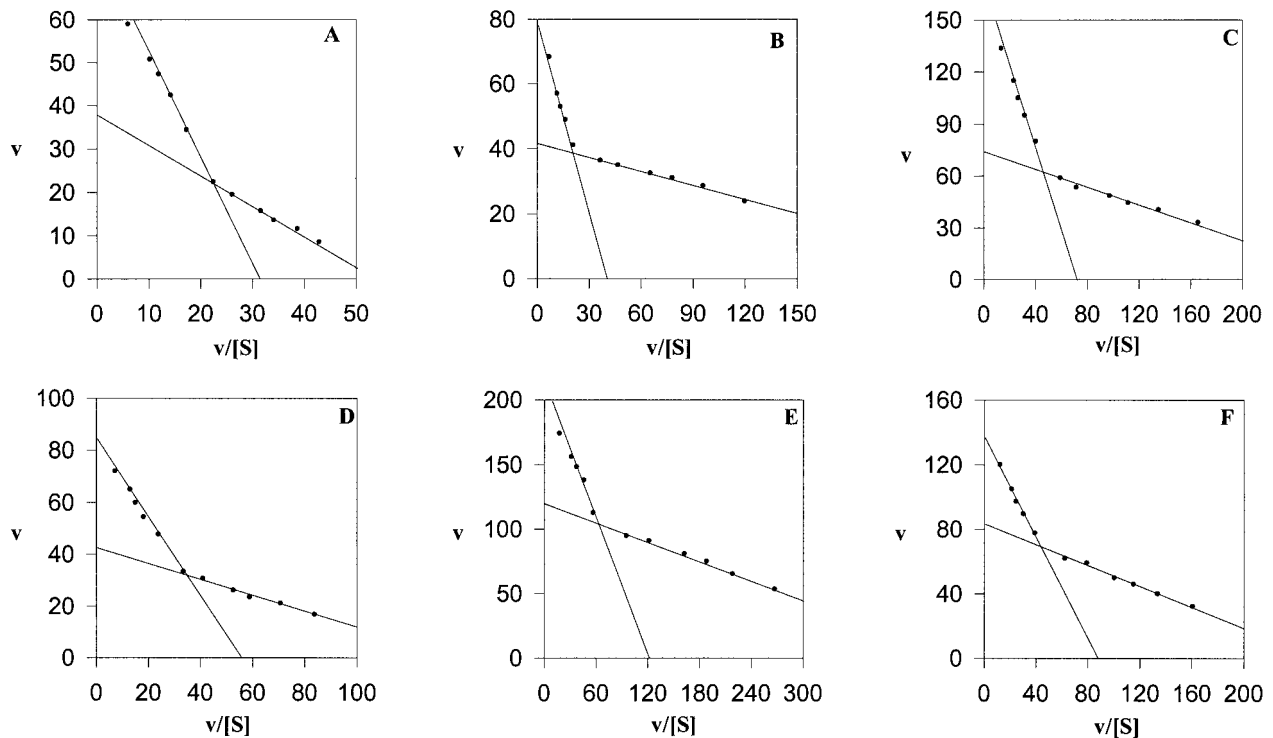


Figure 3 Typical Eadie-Hofstee plots for the rat heart membrane-bound fraction of BChE for (A) control male, (B) control female, (C) diabetic male, (D) diabetic female, (E) insulin-treated diabetic male and (F) insulin-treated diabetic female rats. Experimental details are given in the text. For determination of substrate kinetics of BChE, BCTI was used as the substrate over a concentration range of 0.2–10 mM. The ordinate represents the reaction velocity (v), while the abscissa represents the $v/[S]$ ratios. Reaction velocity = nmol/min per mg protein. $v/[S]$ = reaction velocity divided by the corresponding substrate concentration.

Table 5 Substrate kinetics (K_m , nM; V_{max} , nmol/min per mg protein) of soluble and membrane-bound form of cardiac BChE in male and female rats. Results are expressed as means \pm S.E.M. of the number of independent observations indicated in parentheses

			Component I		Component II	
Sex	Group		K_m	V_{max}	K_m	V_{max}
Fraction of heart						
Soluble						
Male	Control (8)		0.31 ± 0.03	6 ± 0.3	2.3 ± 0.2	17 ± 1.5
	Diabetic (8)		$0.17 \pm 0.01^{**}$	$17 \pm 0.9^{**}$	2.3 ± 0.1	$53 \pm 3.0^{**}$
	Insulin-treated diabetic (11)		$0.19 \pm 0.01^{**}$	$41 \pm 3.1^{**b}$	$1.5 \pm 0.1^{**b}$	$87 \pm 2.6^{**b}$
Female	Control (8)		0.25 ± 0.03	23 ± 1.3	2.2 ± 0.1	54 ± 1.9
	Diabetic (8)		0.26 ± 0.03	24 ± 1.8	$1.7 \pm 0.1^*$	48 ± 2.0
	Insulin-treated diabetic (9)		0.18 ± 0.02	$43 \pm 3.9^{**b}$	$1.6 \pm 0.1^{**}$	$82 \pm 3.5^{**b}$
Membrane-bound						
Male	Control (6)		0.59 ± 0.09	39 ± 4.2	2.5 ± 0.2	81 ± 4.1
	Diabetic (8)		$0.24 \pm 0.03^{**}$	$69 \pm 3.1^{**}$	2.1 ± 0.1	$161 \pm 6.1^{**}$
	Insulin-treated diabetic (11)		$0.35 \pm 0.04^{*a}$	$120 \pm 4.9^{**b}$	$1.6 \pm 0.2^{*a}$	$206 \pm 10.6^{**b}$
Female	Control (6)		0.18 ± 0.01	40 ± 0.8	1.9 ± 0.1	84 ± 3.8
	Diabetic (8)		$0.36 \pm 0.03^*$	$64 \pm 4.3^{**}$	1.8 ± 0.1	$108 \pm 7.4^*$
	Insulin-treated diabetic (9)		$0.28 \pm 0.03^*$	$88 \pm 7.8^{**a}$	$1.5 \pm 0.1^{*a}$	$143 \pm 10.1^{**a}$

* $P < 0.05$ and ** $P < 0.005$ compared with corresponding control; $^aP < 0.05$ and $^bP < 0.005$ compared with corresponding diabetic value.

components of the soluble form of cardiac BChE when compared with the control females. After insulin treatment, the V_{\max} of component I increased by 85% ($P < 0.005$) and 78% ($P < 0.005$) as compared with the control and diabetic females respectively. A similar picture was true for the V_{\max} of component II. After insulin treatment, the V_{\max} of component II increased by 53% ($P < 0.005$) and 71% ($P < 0.005$) as compared with the control and diabetic female rats respectively.

In the diabetic male rats, the K_m of component I of the membrane-bound form of cardiac BChE decreased by 59% ($P < 0.005$) when compared with the control males. The V_{\max} of both components of the membrane-bound form of cardiac BChE increased by 74% ($P < 0.005$) and 99% ($P < 0.005$) respectively as compared with the control male rats. After insulin treatment, the K_m of component I of the membrane-bound form of cardiac BChE increased by 47% ($P < 0.05$) when compared with the diabetic males, but was still lower by 40% ($P < 0.05$) when compared with the control males. Interestingly, after insulin treatment, the K_m of component II decreased by 34% ($P < 0.05$) and 24% ($P < 0.05$) as compared with the control and diabetic males respectively. The V_{\max} of components I and II increased further by 75% ($P < 0.005$) and 28% ($P < 0.005$) after insulin treatment respectively when compared with the diabetic male rats. The V_{\max} values of components I and II remained higher by 205% ($P < 0.005$) and 154% ($P < 0.005$) in the insulin-treated males respectively when compared with the control males.

The influence of the diabetic and insulin-treated diabetic conditions was dramatically different on BChE activities in the membrane-bound fraction of the female rat heart as compared with males. In the control group, the K_m of component I was about three times lower compared with the control males, while the V_{\max} was comparable in both sexes. Also for component II, the K_m was lower (22%) in control females as compared with the control males, while the V_{\max} values were comparable in both sexes. In the diabetic state, K_m and V_{\max} of component I almost doubled while the V_{\max} of component II increased by 29% ($P < 0.05$). After insulin treatment, the K_m of component I decreased somewhat but was still higher (50%, $P < 0.05$) than the control females. After insulin treatment, the K_m of component II decreased by 21% ($P < 0.05$) and 16% ($P < 0.05$) respectively when compared with the control and diabetic female groups. The V_{\max} of component I further increased after insulin treatment by 38% ($P < 0.05$) as compared with the diabetic female rats and remained 2.2-fold ($P < 0.005$) higher as compared with the control female rats. A similar pattern was obtained for the V_{\max} of component II. However, the extent of increase was much less compared with the corresponding males (Table 5).

In brief, the variable effects of the diabetic and insulin-treated diabetic conditions were observed on K_m values in both fractions of both sexes. The value of V_{\max} in general

increased in the diabetic condition in both fractions of both sexes; this further increased in the insulin-treated diabetic condition. In general, the magnitude of increase was higher in the males when compared with the females.

Discussion

In the present study, the alloxan-induced diabetic condition was confirmed by polyuria, glycosuria, hyperglycemia and loss of body weight. The present insulin treatment regime was able to control these characteristics of diabetes.

Sex differences in serum and cardiac BChE activity were observed. The sex difference in the BChE activity was more prominent in the serum and soluble fraction of rat heart when compared with the cardiac membrane-bound fraction. Sex differences in plasma and liver BChE activity have been reported previously (Lamartiniere 1986, Oreskovic & Kunec-Vajic 1992). It has been suggested that the sex difference is caused by estrogen, which at the level of the pituitary gland regulates the pattern of secretion of growth hormone (GH), which in turn acts on the hepatocytes and promotes the synthesis of BChE (Cammisa *et al.* 1988). However, the effect of GH on the synthesis of BChE is controversial (Lamartiniere 1986, Cammisa *et al.* 1988).

In the diabetic male rats, an increase of the BChE activity in the serum, soluble and membrane-bound fractions of the heart was evident when compared with the control males; while, in the diabetic females, the increase in BChE activity was evident only in the membrane-bound fraction of the heart when compared with the control females. The BChE activity was found to be increased in serum, soluble and membrane-bound fractions of the heart in the insulin-treated groups of both sexes.

It has been shown that diabetic rat hearts do not respond to conditions of high stress when compared with controls (Tahiliani & McNeill 1986). Cardiac muscle cells in the streptozotocin-induced 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 were noted (Seager *et al.* 1984). A marked increase in both lysosomes and lipid droplets was apparent (Seager *et al.* 1984).

As has been mentioned in the Introduction, the source of the high levels of serum BChE under diabetic conditions is not known. The effect of diabetes on cardiac BChE has not been studied so far despite high BChE levels in the heart. For easy comparison between the BChE of serum and heart we studied the substrate kinetics of this enzyme in both tissues.

Except for the increase in the K_m of component II of serum BChE in the diabetic females, no statistically

significant changes were evident in serum BChE in the diabetic and insulin-treated diabetic female rats. In males, a high K_m of component II of serum BChE was also evident. In diabetic males, the increase in the V_{max} of component I was of lesser magnitude (65% increase) than the increase in the V_{max} of component II (144% increase). A similar picture was also observed in the insulin-treated diabetic male rats. Thus the data indicated that the increase in the V_{max} of component II may be a key player for high serum BChE activity in the diabetic and insulin-treated diabetic males. Interestingly, the K_m of component II of the soluble and membrane-bound forms of cardiac BChE was comparable with the K_m of component II of serum BChE.

When considering the cardiac BChE data in terms of ratios of specific activity of soluble to membrane-bound forms, the ratio of the soluble to membrane-bound form of the enzyme was 0.26 in control males but was much higher, i.e. 0.68, in the control females. In the diabetic males, the ratio increased slightly, e.g. 0.33, and the trend toward 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).

From the activity data, substrate kinetics of serum and cardiac BChE and the ratio of soluble to membrane-bound forms of cardiac BChE activity, it can be hypothesized that under diabetes-induced cardiac pathology the proportion of the soluble form of cardiac BChE increases. High levels of the soluble form of cardiac BChE could be a possible source of the increased level of serum BChE under the diabetic condition. Increase in cardiac BChE activity in diabetic and insulin-treated diabetic male rats could be a compensatory mechanism. No increase was found in the cardiac BChE activity in diabetic female rats, which could be the reason for the high incidence of mortality due to cardiovascular causes in diabetic females.

At present we are able to control the physiological parameters of diabetes by insulin treatment of diabetic rats. Insulin treatment was not able to improve most of the diabetes-induced alterations in BChE from all the sources studied. Insulin treatment is known to control blood sugar levels but is not able to rectify all the lipid defects (Romano *et al.* 1997). Ido *et al.* (1997) have shown that not all the maladies of diabetes can be corrected by insulin treatment.

In conclusion, basic sex differences were observed in serum and cardiac BChE in rats. The effect of diabetes on serum and cardiac BChE was also sex dependent. Insulin was not able to rectify the diabetes-induced abnormalities in serum and cardiac BChE. The heart could be one of the possible sources of the increased level of serum BChE. Alterations in cardiac BChE could be one of the reasons behind the high incidences of coronary heart disease and congestive heart failure in diabetics.

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