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Effect of Alloxan Diabetes and Subsequent Insulin Treatment on Temperature Kinetics Properties of Succinate Oxidase Activity in Rat Kidney Mitochondria

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Abstract. Early and late effects of alloxan diabetes and subsequent treatment with insulin on the temperature kinetics properties of succinate oxidase (SO) activity in rat kidney mitochondria were examined. In diabetic animals SO activity increased significantly and the increase was more pronounced at the late stage. Insulin treatment partially restored SO activity. However, the effect was temperature-dependent. In diabetic animals the energy of activation in the low temperature range (E_L) increased significantly while that in the high temperature range (E_H) decreased. The latter seems to be responsible for improving catalytic efficiency in the diabetic state. Insulin treatment normalized E_H only in the 1-month diabetic group. The phase transition temperature (Tt), decreased in diabetic animals. Insulin treatment caused an increase beyond the control value in Tt in 1-month diabetic animals. The results suggest that insulin status-dependent modulation of SO activity is a complex process.

Key words: Alloxan diabetes — Succinate oxidase — Insulin status — Diabetic modulation — Arrhenius kinetics — Kidney mitochondria

Introduction

Diabetic nephropathy, a major cause of diabetes-related morbidity and mortality, affects about 15–25% of all type 1 diabetic patients and 20–40% of all patients with type 2 diabetes (Ascic-Buturovic, Surkovic & Heljic, 2005; Jacobsen, 2005; Jawa, Kcomt & Fonseca, 2004; Jensen, Ostergaard & Flyvbjerg, 2005). Diabetic nephropathy leads to structural and functional changes in the kidney (Bohlender et al., 2005; Jawa, Kcomt & Fonseca, 2004). Earlier studies from our laboratory have shown that cathepsin D activity in the kidneys of streptozotocin (STZ) diabetic rats decreased and insulin treatment increased the activity beyond control values (Nerurkar, Satav & Katyare 1988). Alloxan diabetes brought about a significant decrease in the Na^+,K^+-ATPase activity in rat kidney with a fourfold increase in the Km and a 68% reduction in Vmax (Kumthekar & Katyare, 1992). We also observed that insulin status significantly influenced the oxidative energy metabolism of rat kidney mitochondria. Rates of substrate oxidation were differentially affected by STZ diabetes and subsequent treatment with insulin (Katyare & Satav, 2005). Under these conditions, succinate dehydrogenase (SDH) activity and content of cytochromes changed only at the late stage of diabetes. Thus, only in 1-month diabetic animals did SDH activity increase, by about 55%, and this could be partially controlled by insulin treatment (Katyare and Satav, 2005). In parallel studies, we also found that alloxan diabetes and subsequent treatment with insulin significantly changed the kinetics properties of FoF1 adenosine triphosphatase (ATPase) in the liver and kidney mitochondria (Patel & Katyare, 2006a, 2006b). The foregoing results thus point out that the electron transport system of kidney mitochondria is significantly influenced by insulin status.

The enzyme system succinate oxidase (SO) spans a major portion of the electron transport chain from SDH to cytochrome oxidase. Examination of the kinetics properties of SO as influenced by insulin status can thus provide broad-based, deeper insights into the overall effects of insulin status on functional aspects of the electron transport system. The present report summarizes these aspects.

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Materials and Methods

Chemicals

Bovine serum albumin (BSA) fraction V, 1,6 diphenyl-1,3,5-hexatriene (DPH) and sodium salt of ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma (St. Louis, MO). Sodium succinate hexahydrate was purchased from SRL (Mumbai, India). Silica gel G was from Merck (Darmstadt, Germany). Neutral protamine Hagedorn (NPH) insulin (40 U/ml) was obtained from Lilli (Fegersheim, France). All other chemicals were of analytical-reagent grade and were purchased locally.

Animals

Adult male albino rats of Charles-Foster strain weighing 200–250 g were used. The animals were fasted overnight and the diabetic state was induced by injecting subcutaneously (s.c.) 12 mg alloxan/100 g body weight (Dave & Katyare, 2002; Kumthekar & Katyare, 1992; Satav, Dave & Katyare, 2000). Alloxan solutions were prepared freshly in saline. The controls received an equivalent volume of the saline vehicle.

Experiments were carried out at the end of 1 week or 1 month of induction of diabetes to ascertain the early-onset and long-term effects (Katyare & Satav, 2005; Park & Drake, 1982; Satav & Katyare 2004). The diabetic state was confirmed in terms of hyperglycemia, polyuria and glucosuria as detailed previously (Katyare & Satav, 2005; Kumthekar & Katyare, 1992; Nerurkar, Satav & Katyare, 1988; Satav & Katyare, 2004). For 1-week studies the diabetic animals received insulin from the fifth day of induction of diabetes for 3 consecutive days and for 1-month studies the diabetic animals received insulin starting from the fourth week of induction of diabetes for 7 consecutive days at a dose of 0.8 U NPH insulin/100 g body weight twice daily (around 7:00 AM and 6:00 PM) by the s.c. route (Dave & Katyare, 2002; Kumthekar and Katyare, 1992; Satav, Dave & Katyare, 2000).

Isolation of Mitochondria

Isolation of kidney mitochondria was essentially according to the procedures described earlier with some modifications. The isolation medium consisted of 0.25 M sucrose, 10 mM Tris-HCl buffer (pH 7.4) and 1 mM EDTA; 250 μg BSA/ml of isolation medium was included (Katyare et al., 1977; Satav & Katyare, 1991).

SO Assay

Measurement of SO activity was carried out polarographically using a Clarke-type oxygen electrode. The assay medium (final volume 1.6 ml) contained 50 mM potassium phosphate buffer (pH 7.4), 0.4 mM each of $CaCl_2$ and $AlCl_3$ (Dave, Billimoria & Katyare, 1989) and a saturating amount of sodium succinate (10 mM). The measurements were carried out over the temperature range 5–53°C with an increment of 4°C at each step. The activity v, is expressed as nmole O_2/min/mg protein.

Lipid Analysis

The extraction of mitochondrial lipids/phospholipids, isolation of phospholipid classes by thin layer chromatography (TLC), estimation of cholesterol (CHL) and phospholipid phosphorus and determination of membrane fluidity were according to the procedures described (Bartlett, 1954; Pandya, Dave & Katyare, 2001; Skipski et al., 1967; Zlatkis, Zak & Boyle, 1953). Contents of individual phospholipid classes were computed by multiplying the total phospholipid (TPL) content by the percent composition (Pandya et al., 2001).

Table 1. Effect of alloxan diabetes and insulin treatment on SO activity of rat kidney mitochondria

Group	Treatment	Activity (nmole O_2/min/mg protein) 25°C	37°C
1 week	Control (8)	10.9 ± 0.4	57.0 ± 1.6
	Diabetic (6)	34.2 ± 1.8*	83.8 ± 2.8*
	Diabetic + insulin (8)	25.9 ± 1.0*,+	54.1 ± 1.3+
1 month	Control (6)	9.4 ± 0.8	54.5 ± 1.4
	Diabetic (6)	50.5 ± 2.6*	115.8 ± 7.6*
	Diabetic + insulin (8)	14.2 ± 0.5*,+	51.4 ± 1.4+

The results are given as mean ± standard error of the mean, with the number of independent observations indicated in parentheses.
*$P < 0.001$ compared to the corresponding control.
+$P < 0.001$ compared to the corresponding diabetic.

Data Analysis

The data on temperature kinetics were computer-analyzed using Sigma Plot version 6.1 for determination of energies of activation in the low and high temperature ranges (E_L and E_H, respectively) and phase-transition temperature (Tt) according to methods described previously (Dave & Katyare, 2002; Dave, Syal & Katyare, 1999; Dixon & Webb, 1979; Patel et al., 1999).

The regression analysis was carried out across the groups using Jandel Sigmastat Statistical Software, version 2.0 (Jandel Corporation, San Rafael, CA).

Estimation of protein was according to Lowry et al. (1951) using BSA as the standard.

Statistical evaluation of the data was by Students' *t*-test.

Results and Discussion

The effects of the diabetic state and insulin treatment were ascertained by determining the standard parameters such as blood sugar level, polyuria, glucosuria and renal hypertrophy. These parameters were in conformity with earlier reported observations (Katyare & Satav, 2005; Kumthekar & Katyare, 1992; Nerurkar, Satav & Katyare 1988; Satav & Katyare, 2004) and hence are not included in the present report.

In preliminary studies, we checked the effect of insulin status on SO activity at early and late stages of diabetes. The data are given in Table 1. Measurements at 25°C revealed that SO activity had increased significantly in both 1-week as well as 1-month diabetic animals (3.14- and 5.37-fold increase, respectively). Treatment with insulin brought the activity closer to control values only in the 1-month diabetic animals. When the measurements were carried out at 37°C, the increase in activity amounted to 1.47- and 2.12-fold

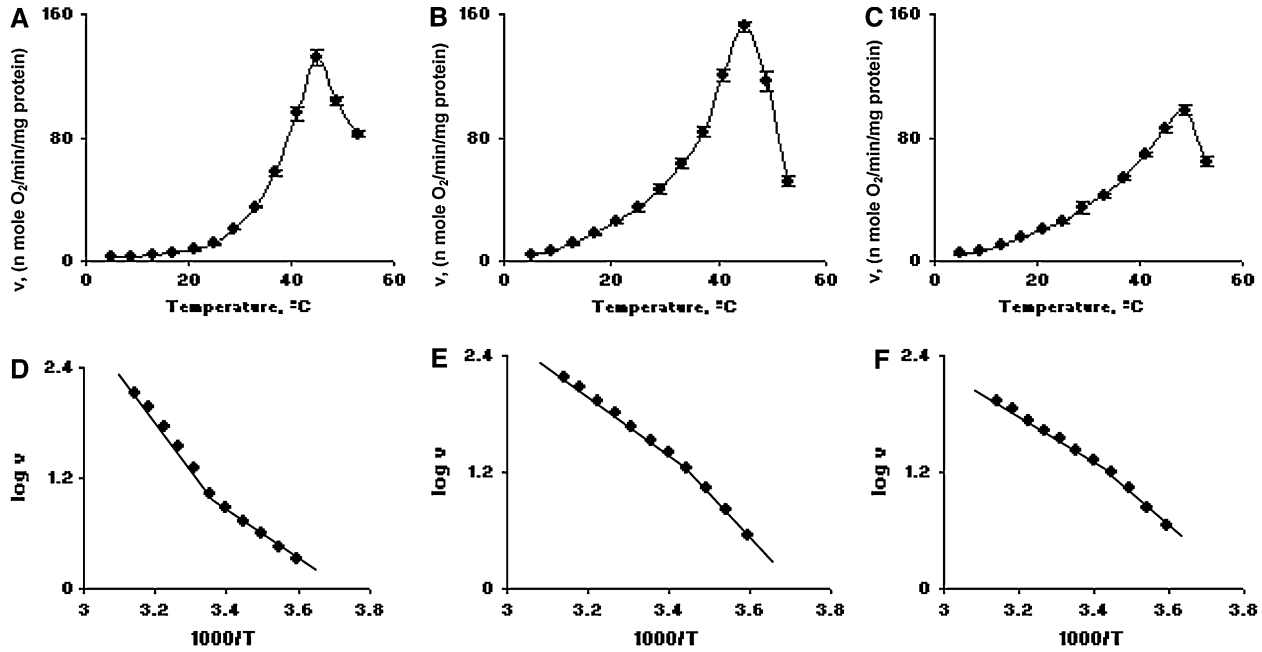

Fig. 1. Plots depicting dependence of enzyme activity on temperature and the corresponding Arrhenius plots for 1-week groups. In the temperature curves, the enzyme activity v (nmole O_2/min/mg protein), on the ordinate is plotted *vs* temperature (°C) on the abscissa. A–C represent the control, diabetic and insulin-treated diabetic groups, respectively. In Arrhenius plots log of v on the ordinate is plotted vs 1000/T on the abscissa, where v and T represent, respectively, the activity at corresponding absolute temperature T (°C + 273.18). D–F represent the control, diabetic and insulin-treated diabetic groups, respectively. Each data point represents the mean of six to eight independent experiments, as indicated in the tables. The standard error of the mean for the individual points ranged from 2–5%.

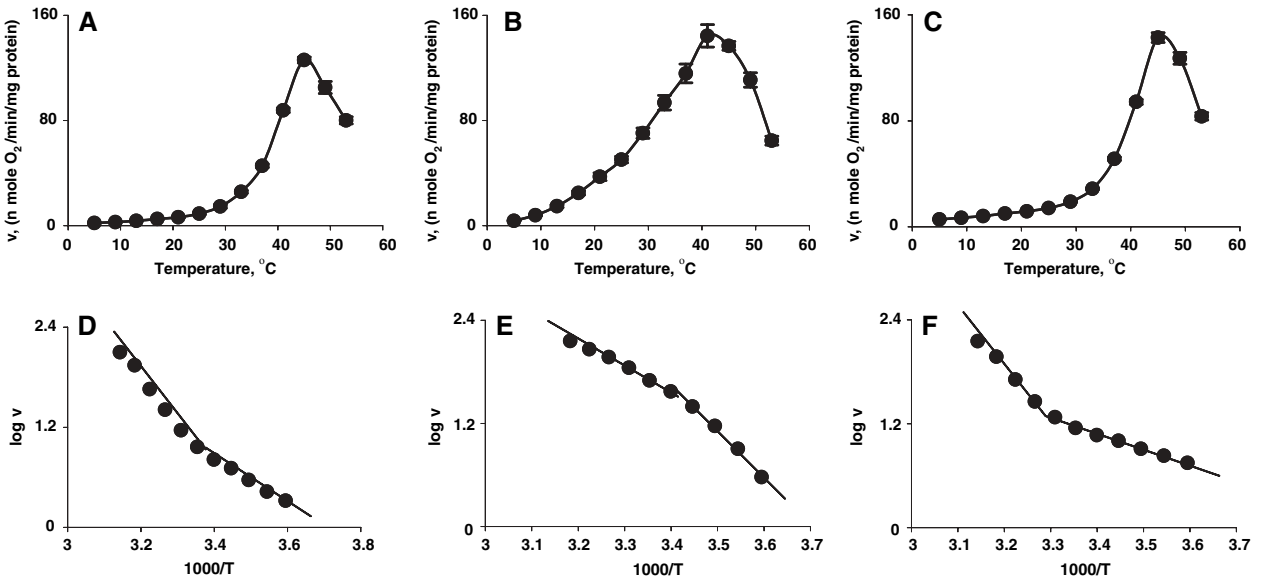

Fig. 2. Plots depicting dependence of enzyme activity on temperature and the corresponding Arrhenius plots for 1-month groups. In the temperature curves, the enzyme activity v (nmole O_2/min/mg protein), on the ordinate is plotted *vs* temperature (°C) on the abscissa. A–C represent the control, diabetic and insulin-treated diabetic groups, respectively. In Arrhenius plots log of v on the ordinate is plotted vs 1000/T on the abscissa, where v and T represent, respectively, the activity at corresponding absolute temperature T (°C + 273.18). D–F represent the control, diabetic and insulin-treated diabetic groups, respectively. Each data point represents the mean of six to eight independent experiments, as indicated in the tables. The standard error of the mean for the individual points ranged from 2–5%.

Table 2. Effect of alloxan diabetes and insulin treatment on Arrhenius kinetics properties of SO of rat kidney mitochondria

Group	Treatment	Energy of activation (kJ/mole)		Phase transition temperature (Tt,°C)
		E_L	E_H	
1 week	Control (8)	54.5 ± 3.0	101.9 ± 4.5	22.9 ± 0.88
	Diabetic (6)	97.8 ± 3.4**	58.8 ± 1.3**	15.8 ± 0.67**
	Diabetic + insulin (8)	77.0 ± 3.0$^{**, ‡}$	46.4 ± 1.1$^{**, ‡}$	18.8 ± 0.46$^{*, †}$
1 month	Control (6)	52.2 ± 2.3	111.4 ± 4.6	24.4 ± 0.50
	Diabetic (6)	108.6 ± 4.9**	51.5 ± 1.5**	19.0 ± 0.80**
	Diabetic + insulin (8)	39.5 ± 1.5$^{**, ‡}$	101.9 ± 2.8‡	28.0 ± 0.55$^{*, ‡}$

The results are given as mean ± standard error of the mean, with the number of independent observations indicated in parentheses.
$^{*}P < 0.002$ and $^{**}P < 0.001$ compared to the corresponding control.
$^{†}P < 0.01$ and $^{‡}P < 0.001$ compared to the corresponding diabetic.

respectively, in 1-week and 1-month diabetic animals. Insulin treatment completely restored the activity in both diabetic groups (Table 1).

These temperature-dependent differential effects (Table 1) prompted us to examine in detail the temperature dependence of the activity under different experimental conditions. The typical activity vs temperature plots and the corresponding Arrhenius plots for the 1-week and 1-month groups are shown in Figures 1 and 2.

Consistent with the data in Table 1, at any given temperature the activities were high in the diabetic groups (Figs. 1A–C and 2A–C). Insulin treatment had an apparent generalized restorative effect. However, the important and interesting feature was the shift in the optimum temperature. In control groups, the optimum temperature was 45°C, which did not change at the early stage of diabetes but decreased to 41°C at the late stage. Insulin treatment shifted the optimum temperature to 49°C in the 1-week diabetic group whereas in 1-month diabetic animals it was restored to 45°C (e.g., Figs. 1 and 2).

The differences in temperature dependence of the activity among different groups became evident when the data were transformed in the corresponding Arrhenius plots (Figs. 1D–F and 2D–F).

Thus, the interesting feature of the Arrhenius plots for the control group was that the energy of activation in the low temperature range (E_L) was low while that in the high temperature range (E_H) was high (Figs. 1D and 2D). In other words, the pattern was opposite to what is commonly noted for most of the enzyme systems, i.e., high values of E_L and low values of E_H (Dave & Katyare, 2002; Dave, Syal & Katyare, 1999; Dixon & Webb, 1979; Patel et al., 1999). The diabetic state reversed the pattern (Figs. 1D-F and 2D–F). Insulin treatment was unable to restore the pattern to normal in the 1-week group (Fig. 1D–F). However, in the 1-month diabetic group, the pattern was restored to normal following insulin treatment (Fig. 2D–F).

The values for E_L, E_H and Tt determined from the Arrhenius plots are given in Table 2. Thus, in the control groups the values of E_L and E_H were around 52–55 and 102–111 KJ/mole, with phase transition occurring at around 23–24°C. In both diabetic groups, E_L almost doubled whereas E_H decreased to about half the control value. In diabetic animals, Tt, decreased significantly with the effect being more marked in the early stage. Treatment with insulin in the 1-week diabetic group partially lowered E_L and brought about a further decrease in E_H. In 1-month diabetic animals E_L decreased further while E_H became comparable to the control group; T_t was not restored to control levels. In the 1-week group insulin treatment elevated T_t, although it was still lower than that in the controls. By contrast, in the 1-month group, insulin treatment caused an increase in T_t beyond the control level. The low values of T_t in the 1-week diabetic group treated with insulin seem to be paradoxical, especially in view of the fact that the fatty acid desaturase activity and unsaturation index decrease in diabetes (Coste et al., 1999; Kuwahara et al., 1997).

The interesting feature of the present studies was the increase in SO activity in diabetic animals and its suppression by insulin treatment. The enzyme SDH is responsible for initiating the process of electron transfer, which is a rate-limiting step in SO activity (Singer, Gutman & Massey, 1973). We have earlier noted that SDH activity increased marginally only in the 1-month diabetic group and insulin treatment could only partly lower this activity (Katyare & Satav, 2005). Therefore, it is unlikely that the observed increase in SO in the diabetic or insulin-treated diabetic animals is attributable to this factor. SDH is known to be activated by several physiological activators, which include ATP, reduced nicotinamide adenine dinucleotide (NADH), coenzyme Q (CoQ) and inorganic phosphate (P_i) (Singer, Gutman & Massey, 1973). Insulin status-dependent changes in the contents of CoQ and of cytochromes in mitochondria have been demonstrated (Ferreira et al.,

Table 3. Effects of alloxan diabetes on total phospholipids (TPL), cholesterol (CHL), TPL/CHL ratio, and fluorescence polarization in rat kidney mitochondria

Groups	Treatment	TPL (μg/mg protein)	CHL (μg/mg protein)	TPL/CHL (mole:mole)	Fluorescence polarization (p)
1 week	Control (8)	266.4 ± 3.35	94.0 ± 3.86	1.43 ± 0.06	0.206 ± 0.001
	Diabetic (6)	254.7 ± 2.58	105.4 ± 3.26**	1.21 ± 0.04*	0.272 ± 0.004***
	Diabetic + insulin (8)	246.1 ± 5.06*	84.8 ± 3.61‡	1.46 ± 0.04‡	0.123 ± 0.002$^{***, \ddagger}$
1 month	Control (6)	261.8 ± 3.05	90.4 ± 3.21	1.46 ± 0.06	0.210 ± 0.002
	Diabetic (6)	131.4 ± 6.57***	146.7 ± 7.83***	0.45 ± 0.03***	0.265 ± 0.010***
	Diabetic + insulin (8)	205.6 ± 10.88$^{***, \ddagger}$	113.9 ± 3.54***,†	0.90 ± 0.04$^{***, \ddagger}$	0.238 ± 0.004$^{***, \ddagger}$

The experimental details are given in Table 1.
The results are as given as mean ± standard error of the mean, with the number of independent observations indicated in parentheses.
$^{*}P < 0.01$, $^{**}P < 0.05$ and $^{***}P < 0.001$ compared to the corresponding control.
$^{\dagger}P < 0.01$ and $^{\ddagger}P < 0.001$ c0ompared to the corresponding diabetic.

Table 4. Effects of alloxan diabetes and insulin treatment on phospholipid composition in rat kidney mitochondria

	Composition (% of total)					
	1 week			1 month		
Phospholipid class	Control (8)	Diabetic (6)	Diabetic + insulin (8)	Control (6)	Diabetic (6)	Diabetic + insulin (8)
Lyso	2.2 ± 0.05	6.4 ± 0.18****	1.7 ± 0.12$^{***, \ddagger}$	2.0 ± 0.05	3.6 ± 0.17****	1.7 ± 0.03$^{****, \ddagger}$
SPM	7.8 ± 0.19	10.4 ± 0.38****	7.1 ± 0.23$^{*, \ddagger}$	7.5 ± 0.11	10.2 ± 0.53****	10.1 ± 0.42****
PC	38.4 ± 0.88	40.4 ± 0.66	40.6 ± 0.43	39.2 ± 0.63	40.1 ± 0.97	34.8 ± 0.80$^{****, \ddagger}$
PI	1.1 ± 0.20	2.9 ± 0.23****	1.1 ± 0.05‡	1.3 ± 0.28	2.3 ± 0.14**	2.5 ± 0.18****
PS	1.0 ± 0.10	2.4 ± 0.09****	1.3 ± 0.11‡	1.0 ± 0.08	5.0 ± 0.20****	6.8 ± 0.47$^{****, \ddagger}$
PE	34.3 ± 0.62	25.2 ± 0.75****	36.0 ± 0.39$^{*, \ddagger}$	33.6 ± 0.50	26.7 ± 1.33****	32.4 ± 0.48†
DPG	15.2 ± 0.61	12.3 ± 0.18****	12.3 ± 0.31****	15.4 ± 0.49	12.1 ± 0.50****	11.8 ± 0.27****

The results are given as mean ± standard error of the mean, with the number of independent observations indicated in parentheses.
$^{*}P < 0.05$, $^{**}P < 0.02$, $^{***}P < 0.01$ and $^{****}P < 0.001$ compared to the corresponding control.
$^{\dagger}P < 0.002$ and $^{\ddagger}P < 0.001$ compared to the corresponding diabetic.

Table 5. Effects of alloxan diabetes and insulin treatment on phospholipid content in rat kidney mitochondria

	Content (μg/mg protein)					
	1 week			1 month		
Phospholipid class	Control (8)	Diabetic (6)	Diabetic + insulin (8)	Control (6)	Diabetic (6)	Diabetic + insulin (8)
Lyso	5.78 ± 0.20	16.16 ± 0.59***	4.17 ± 0.35$^{**, \dagger}$	5.35 ± 0.17	4.74 ± 0.57	4.15 ± 0.11***
SPM	20.66 ± 0.87	26.53 ± 1.12**	16.89 ± 0.58$^{**, \dagger}$	20.32 ± 0.44	13.62 ± 1.66***	25.11 ± 0.93***,†
PC	101.40 ± 2.06	101.25 ± 3.08	97.89 ± 4.66	106.10 ± 1.32	52.87 ± 4.78***	86.51 ± 2.66***,†
PI	3.05 ± 0.58	7.36 ± 0.67***	2.55 ± 0.19†	3.60 ± 0.57	3.08 ± 0.34	6.10 ± 0.48*,†
PS	2.78 ± 0.29	6.07 ± 0.24***	2.91 ± 0.26†	2.62 ± 0.24	6.52 ± 0.61***	16.96 ± 1.11***,†
PE	90.87 ± 3.50	62.99 ± 1.44***	86.28 ± 4.35†	91.05 ± 1.91	34.27 ± 1.65***	80.57 ± 2.52***,†
DPG	40.59 ± 2.77	31.19 ± 0.64***	29.76 ± 1.59*	41.79 ± 1.71	16.07 ± 1.78***	29.30 ± 0.93***,†

The results are given as mean ± standard error of the mean, with the number of observations indicated in parentheses.
$^{*}P < 0.01$, $^{**}P < 0.002$, and $^{***}P < 0.001$ compared to the corresponding control.
$^{+}P < 0.001$ compared to the corresponding diabetic.

2003; Katyare & Satav, 2005; Satav & Katyare, 2004). However, the magnitude of these changes (Ferreira et al., 2003; Katyare & Satav, 2005; Satav & Katyare, 2004) is not compatible with observed changes in SO activities.

The other interesting feature of the present studies was the uncommon nature of the Arrhenius plots in the control animals and its reversal in the diabetic animals (Figs. 1 and 2). The net effect was that in the diabetic animals the value of E_H (i.e.,

energy of activation in the high temperature range) decreased substantially. As is evident, the phase-transition temperature, T_t, was around 16–19°C. This would mean that the high temperature range covered the physiological temperature of 37°C. Therefore, the direct consequence would be that in the diabetic animals the energy barrier would decrease, which would allow the enzyme system to function more efficiently. This could thus be a possible regulatory mechanism.

SO has been reported to require bulk membrane phospholipids for its activity (Daum, 1985). In view of this, we tried to correlate v, E_L, E_H and T_t with phospholipid components across the experimental groups at early as well as late stages. Since energies of activation and Tt showed significant insulin status-dependant changes, it was also of interest to find out if a correlation with lipid/phospholipid make-up existed.

The data on the effect of alloxan diabetes and subsequent treatment with insulin on lipid/phospholipid profiles and contents are given in Tables 3–5. Thus, the data in Table 3 show that in the early diabetic state the CHL content increased by 11% without any change in the TPL content. Consequently, the TPL/CHL (mole:mole) ratio decreased with a simultaneous decrease in membrane fluidity. Insulin treatment caused about 8% and 10% decreases in TPL and CHL contents respectively, and the TPL/CHL ratio was comparable to the control. Interestingly, however, membrane fluidity increased by insulin treatment. In 1-month diabetic animals TPL content decreased by about 50% whereas CHL content registered a significantly higher increase (62%) compared to the 1-week diabetic group. As is to be expected, there was a drastic decrease in the TPL/CHL ratio and the membrane fluidity decreased. Insulin treatment was only partially able to restore these parameters.

Analysis of phospholipid profiles revealed that the diabetic states resulted in an increased proportion of lysophospholipids (Lyso), sphingomyelin (SPM), phosphatidylinositol (PI) and phosphatidylserine (PS) components. The effect of the diabetic state on the phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) components was opposite; these components decreased. In the 1-week diabetic group insulin treatment was able to restore Lyso, SPM, PI, PS and PE; DPG was not restored. In long-term diabetes, i.e., the 1-month group, only Lyso and PE were restored by insulin treatment. The proportion of the phosphatidylcholine (PC) component was practically unaffected under all the experimental conditions (Table 4). These changes were also reflected in terms of the contents of the individual phospholipids (Table 5).

Regression analysis across the groups revealed that PE showed a negative correlation with E_L ($r = -0.650$) while PC correlated positively with Tt ($r = +0.614$). SO activity v, by itself did not show any correlation with any of the lipid/phospholipid classes. The latter observation is consistent with and substantiates the earlier report that SO has a non-specific requirement for phospholipids (Daum, 1985). The bulk membrane lipids seem to meet this requirement (Daum, 1985).

It has been reported that in experimental diabetic animals and in human diabetic patients the levels of 3,5,3′-triiodothyronine (T_3) and thyroxine (T_4) decreased significantly (Baydas, Karagoz & Meral, 2002; Katovich, Marks & Sninsky, 1993; Radetti et al., 1994; Rodgers, Noble & Taylor, 1994; Rondeel et al., 1992; Tabata et al., 1999). Additionally, the nuclear T_3 receptors in diabetic rat liver and kidney decreased (Jolin, 1987, 1988). It is therefore possible that the changes which we observe here may also relate to insulin status-dependent alterations in the thyroid hormone levels. This interesting possibility, however, needs further exploration. Indeed, in our earlier studies we observed that experimental thyrotoxicosis induced by treatment with T_3 or T_4 resulted in an increase in the energies of activation and a decrease in T_t of SO activity in rat heart mitochondria (Dave, Billimoria & Katyare, 1989).

In conclusion, our present results point out that regulation of SO in diabetes is a complex process. Additionally, our studies show that modulation of temperature kinetics properties may be a regulatory mechanism which improves the catalytic efficiency of the enzyme system.

It may hence be suggested that the observed changes in all the experimental groups except the insulin-treated 1-month diabetic group with lowered values of E_H, T_t as well as E_L, may represent a mechanism to improve catalytic efficiency of the enzyme system under experimental conditions.

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