Chapter 10

Effects of Alloxan-Diabetes and Subsequent Treatment with Insulin on Kinetic Properties of Na^+, K^+-ATPase from Rat Brain Microsomes

Introduction

As described earlier alloxan-diabetes and subsequent insulin treatment significantly and differentially altered the kinetic properties of Na^+, K^+-ATPase and glucose-6-phosphatase (G6Pase) in liver and kidney microsomes. Also, lipid/phospholipid profiles of liver and kidney microsomes changed significantly under these conditions (Chapters 8 and 9 of the Thesis). In brain the G6Pase localized in mitochondria (1, 2). Hence studies were carried out to examine early and late effects of alloxan-diabetes and subsequent insulin treatment on kinetic properties of Na^+, K^+-ATPase from microsomes. The lipid/phospholipid profiles were also examined under these conditions. The results are summarized below.

Materials and Methods

Chemicals

Details of chemicals required, procedure of induction of diabetes, insulin treatment, isolation of microsomes, extraction of lipid/phospholipids, estimation of cholesterol, determination of phospholipid profile and membrane fluidity are as described in Chapter 2 of the Thesis.

Details of ATPase assay are as described in Chapter 8 of the Thesis.

The substrate and temperature kinetics data were analyzed as detailed (Chapter 2 of the Thesis).

Results

In the preliminary studies ATPase activity was determined at room temperature (25 °C) and at the physiological temperature (37 °C). The data are given in Table 1. As can be noted at early stage of diabetes ATPase activity was unchanged; insulin treatment increased the activity beyond control value. At late stage the enzyme activity decreased significantly (34 and 23 % decrease at the two temperatures); following insulin treatment activity at 25 °C was unchanged while at 37 °C the activity was restored partially. The activity ratios were comparable under all experimental conditions (Table 1).

In view of these differential changes in one week and one month groups further experiments were carried out to examine the kinetic behavior of ATPase as a response to change in the substrate i.e. ATP concentration. The data on substrate kinetics were analyzed as detailed (Chapter 2 of the Thesis). The typical substrate saturation and corresponding Eadie-Hofstee plots and Hill plots are shown in Fig. 1 and 2 Panels A-C and D-F respectively. As can be noted, under all experimental conditions the enzyme activity resolved in two kinetically distinguishable components (Fig. 1 and 2 Panels D-F).

Data on Km and Vmax are given in Table 2. Thus, in the control group the Km value of the two components were 0.24-0.26 and 1.86-1.93 mM respectively with corresponding values of Vmax being 9.13-9.33 and 33.98-36.73 units. Early diabetic state resulted increased in Km of component I whereas Km of component II decreased. At late stage Km of component I decreased while the Km of component II was unchanged. The Vmax of component I increased by 52 % at early stage of diabetes whereas at late stage no

Table 1. Effect of alloxan-diabetes and insulin treatment on $Na^+ K^+$ -ATPase activity in rat brain microsomes

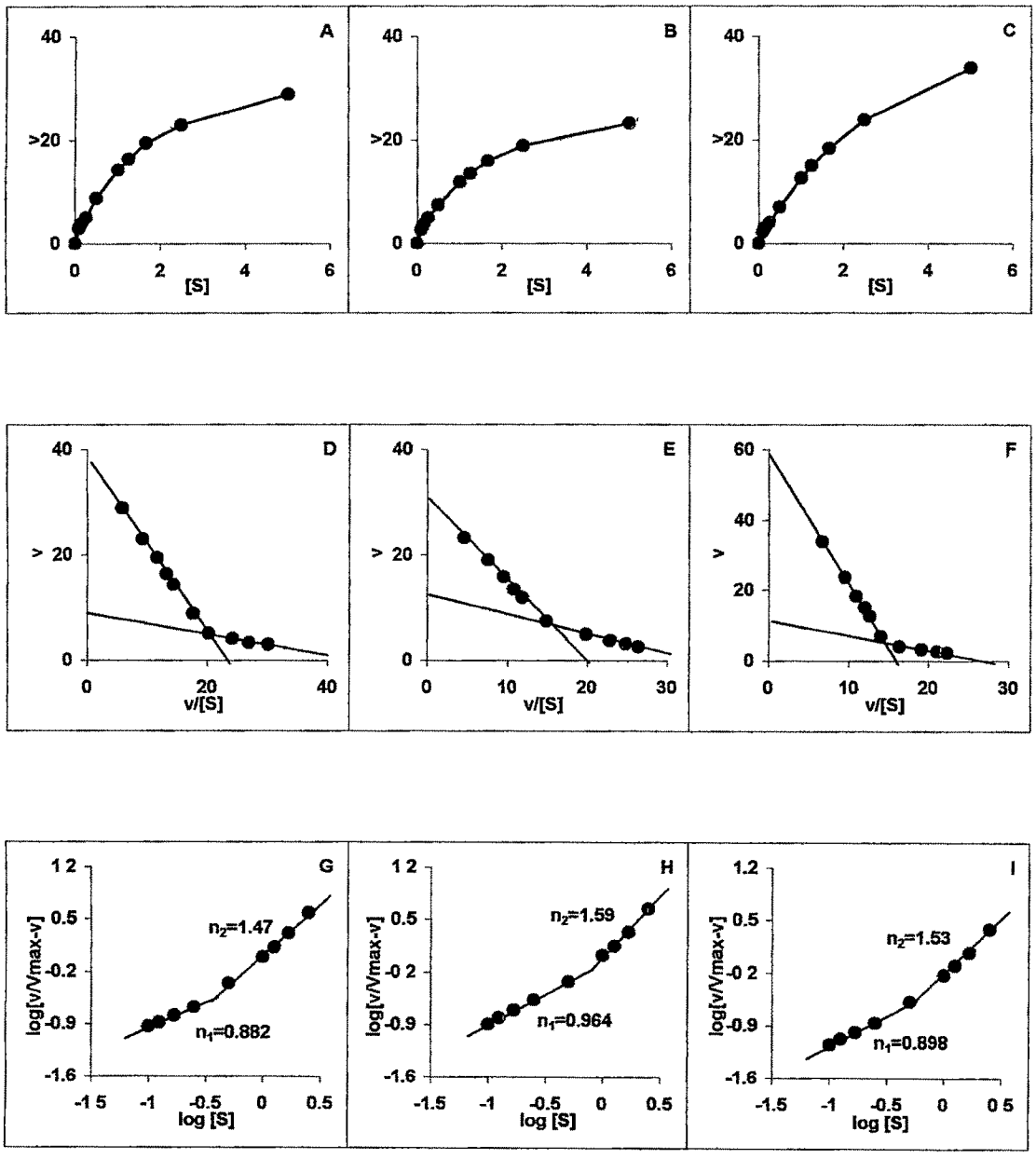
Group	Treatment	Activity (μmole of pi liberated / hr / mg protein)		Activity Ratio
		25 °C	37 °C	
One week	Control	12.65 ± 0.57	24.47 ± 1.16	1.93 ± 0.11
	Diabetic	10.74 ± 0.54	23.60 ± 0.96	2.20 ± 0.12
	Diabetic + Insulin	16.30 ± 0.45[a,§]	30.90 ± 0.66[a,§]	1.90 ± 0.08
One month	Control	12.75 ± 0.52	24.50 ± 0.47	1.92 ± 0.10
	Diabetic	8.43 ± 0.37[a]	18.92 ± 0.66[a]	2.24 ± 0.11
	Diabetic + Insulin	9.47 ± 0.34[a]	21.28 ± 0.42[a,Ψ]	2.25 ± 0.13

The results are given as mean ± SEM of 6-8 independent experiments in each group.

Activity ratio was calculated by: activity at 37 °C/activity at 25 °C

a, $p<0.001$ compared to the corresponding control.
Ψ, $p<0.02$ and §, $p<0.001$ compared to the corresponding diabetic.

Figure 1


A
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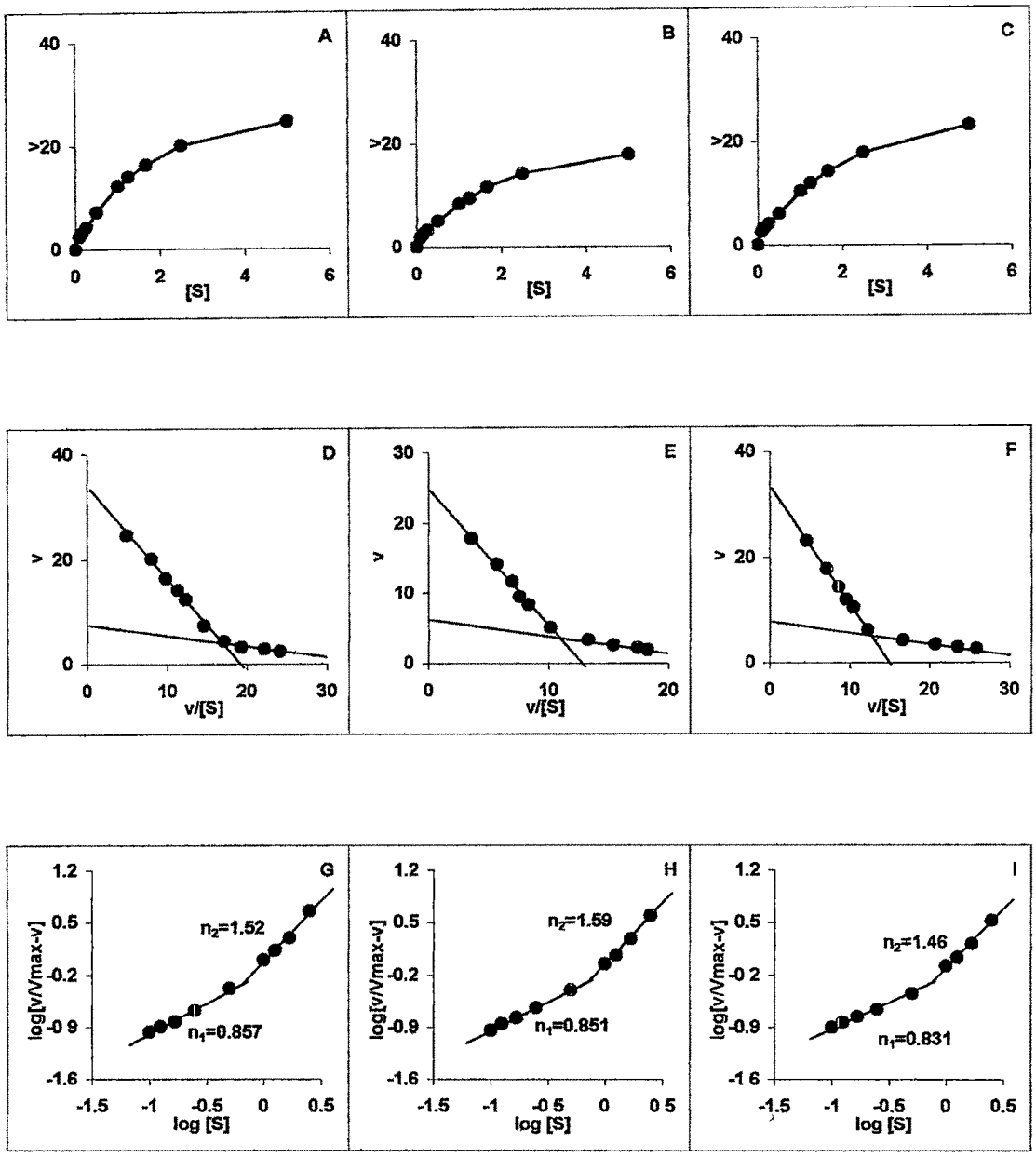
20
10
30
log[v/Vmax-v]
log [S]
1.2
0.5
-0.2
-0.9
-1.6
-1.5
-1
-0.5
0
n_2=1.47
n_1=0.882
n_2=1.59
n_1=0.964
n_2=1.53
n_1=0.898

Figure 2


A
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v/[S]
G
H
I
1.2
0.5
-0.2
-0.9
-1.6
log[v/Vmax-v]
n2=1.52
n1=0.857
n2=1.59
n1=0.851
n2=1.46
n1=0.831
-1.5
-1
-0.5
log [S]

Figure 1. Typical substrate saturation curve (Panels A, B, C), corresponding Eadie-Hofstee (Panels D, E, F) and Hill (Panels G, H, I) plots for Na^+, K^+-ATPase from brain microsomes for one week group. In substrate saturation curve the enzyme activity v on abscissa is plotted versus [S] on ordinate v is the enzyme activity at the given ATP concentration [S]. A, B and C represent to the control, diabetic and insulin-treated diabetic groups. In Eadie-Hofstee plot the enzyme activity v on abscissa is plotted versus v/[S] on ordinate. D, E and F represent to the control, diabetic and insulin-treated diabetic groups. In Hill plot log (v/Vmax-v) on abscissa is plotted versus log [S] on ordinate. The Hill coefficients n_1 and n_2 depict number of ATP molecules bound for the given concentration range of ATP. G, H and I represent to the control, diabetic and insulin-treated diabetic groups. The plots are typical of 6-8 independent experiments in each group.

Figure 2. Typical substrate saturation curve (Panels A, B, C), corresponding Eadie-Hofstee (Panels D, E, F) and Hill (Panels G, H, I) plots for Na^+, K^+-ATPase from brain microsomes for one week group. In substrate saturation curve the enzyme activity v on abscissa is plotted versus [S] on ordinate v is the enzyme activity at the given ATP concentration [S]. A, B and C represent to the control, diabetic and insulin-treated diabetic groups. In Eadie-Hofstee plot the enzyme activity v on abscissa is plotted versus v/[S] on ordinate. D, E and F represent to the control, diabetic and insulin-treated diabetic groups. In Hill plot log (v/Vmax-v) on abscissa is plotted versus log [S] on ordinate. The Hill coefficients n_1 and n_2 depict number of ATP molecules bound for the given concentration range of ATP. G, H and I represent to the control, diabetic and insulin-treated diabetic groups. The plots are typical of 6-8 independent experiments in each group.

Table 2. Effect of alloxan-diabetes and insulin treatment on substrate kinetics properties of Na^+, K^+-ATPase in rat brain microsomes

Group	Treatment	Component I		Component II	
		Km	Vmax	Km	Vmax
One week	Control	0.24 ± 0.01	9.13 ± 0.15	1.86 ± 0.04	36.73 ± 0.65
	Diabetic	0.42 ± 0.02[b]	13.88 ± 0.47[b]	1.64 ± 0.03[b]	33.15 ± 0.71[a]
	Diabetic + Insulin	0.43 ± 0.01[§]	12.73 ± 0.65[b]	3.39 ± 0.04[b,§]	54.12 ± 0.47[b,§]
One month	Control	0.26 ± 0.01	9.33 ± 0.23	1.93 ± 0.05	33.98 ± 0.40
	Diabetic	0.44 ± 0.01[b]	9.42 ± 0.25	1.99 ± 0.03	24.65 ± 0.21[b]
	Diabetic + Insulin	0.26 ± 0.01[§]	8.67 ± 0.31	1.99 ± 0.07	30.58 ± 0.82[a,§]

The Km (mM) and Vmax (μmole of pi liberated / hr / mg protein) values were calculated as described in the text.

The results are given as mean ± SEM of 6-8 independent experiments. As indicated in the text, the kinetic components represent the potential and the response of the enzyme to increasing concentrations of the substrate.

a, $p< 0.05$; b, $p< 0.01$; c, $p< 0.002$ and d, $p<0.001$ compared to the corresponding control.
ψ, $p< 0.05$ and §, $p<0.001$ compared to the corresponding diabetic.

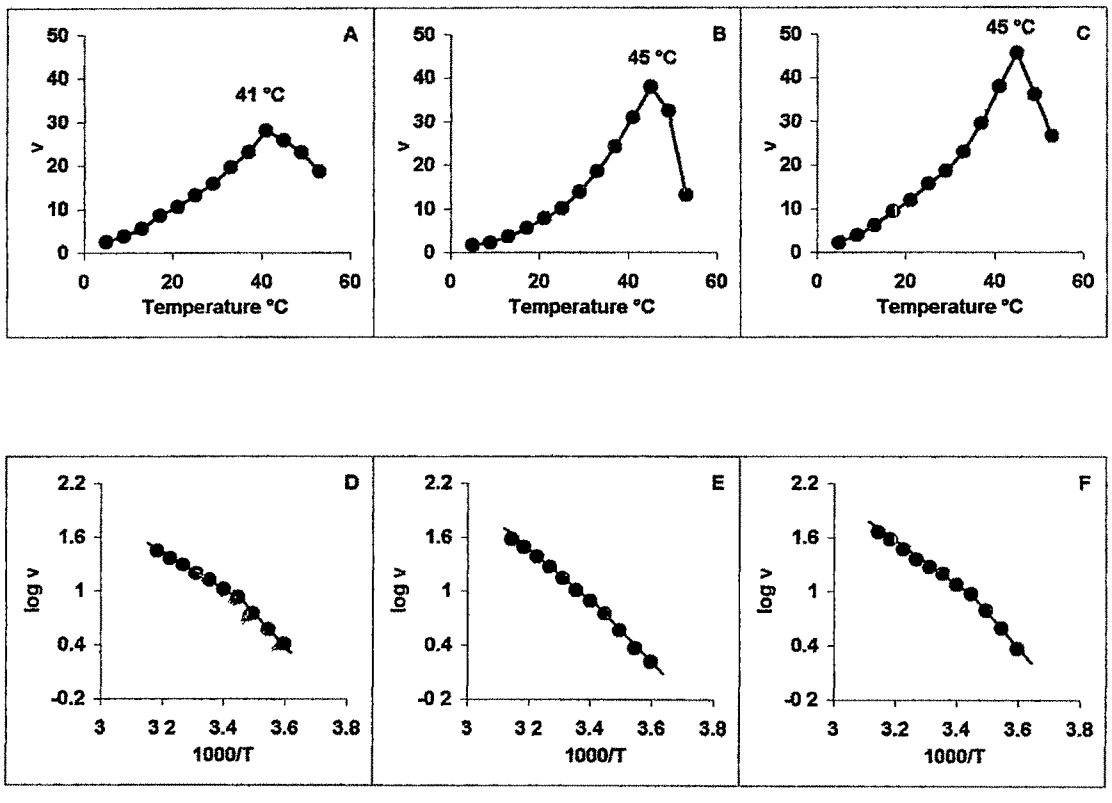
effect was seen. Vmax for component II decreased progressively with diabetes. Insulin treatment in one week diabetic group was ineffective in restoring the Km values for component I whereas Km for component II increased beyond control value. In one month diabetic animals, insulin treatment completely restored the Km for component I. Insulin treatment had partial restorative effect only on the value of Vmax for component II in one month diabetic group (Table 2).

Analysis of the data by Hill plots indicated that up to 0.6 mM ATP concentration one ATP molecule was bound to the enzyme while beyond this concentration two molecules of ATP were bound under all experimental conditions (data not given) Typical Hill plots are shown in Fig. 1 and 2, Panels G-I.

In the next set of experiment the temperature dependence of the enzyme activity was examined. The typical activity versus temperature plots and corresponding Arrhenius plots for early and late effects are given in the Fig. 3 and 4, Panels A-C and D-F respectively. As can be seen, in one week as well as one month groups the activity versus temperature plots in the experimental groups differed considerably from the corresponding controls (Fig. 3 and 4). The optimum temperature in the control group was 41 °C which shifted to 45 °C under all experimental conditions (Fig. 3 and 4 Panels A-C).

The differences were also reflected in terms of corresponding Arrhenius plots (Fig. 3 and 4, Panels D-F). In the control group Arrhenius plots depicted typical biphasic pattern i.e.

Figure 3


A
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Temperature °C
B
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C
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log v
1000/T
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log v
1000/T
F
log v
1000/T

Figure 4

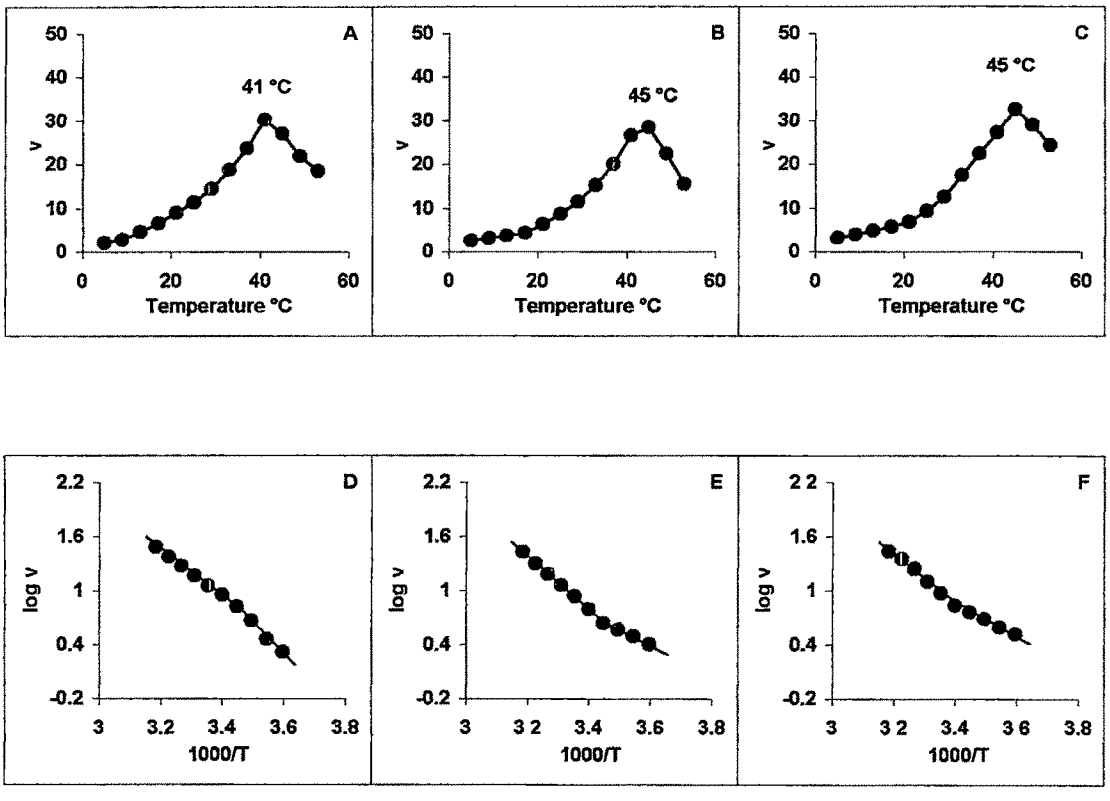
A
41 °C
v
Temperature °C
B
45 °C
v
Temperature °C
C
45 °C
v
Temperature °C
D
log v
1000/T
E
log v
1000/T
F
log v
1000/T

Figure 3. Typical plots depicting dependence of enzyme activity on the temperature and corresponding Arrhenius plots for Na^+, K^+-ATPase from brain microsomes for one week groups. In temperature curves, enzyme activity v on abscissa is plotted verses temperature (°C) on ordinate. A, B and C represent to the control, diabetic and insulin-treated diabetic groups. In Arrhenius plots log of v on ordinate is plotted against 1000/T on abscissa where v and T represent respectively, the activity at corresponding absolute temperature T (temperature in ° Celcius + 273.2). D, E and F represent to the control, diabetic and insulin treated diabetic groups. The plots are typical of 6-8 independent experiments in each group.

Figure 4. Typical plots depicting dependence of enzyme activity on the temperature and corresponding Arrhenius plots for Na^+, K^+-ATPase from brain microsomes for one month groups. In temperature curves, enzyme activity v on abscissa is plotted verses temperature (°C) on ordinate. A, B and C represent to the control, diabetic and insulin-treated diabetic groups. In Arrhenius plots log of v on ordinate is plotted against 1000/T on abscissa where v and T represent respectively, the activity at corresponding absolute temperature T (temperature in ° Celcius + 273.2). D, E and F represent to the control, diabetic and insulin treated diabetic groups. The plots are typical of 6-8 independent experiments in each group.

the energy of activation in high temperature ranges (E_H) was lower than that in low temperature ranges (E_L). In one week experimental groups the Arrhenius pattern was unchanged (Fig. 3 Panel D-F). However, in one month experimental groups the pattern reversed, i.e. energy of activation in high temperature ranges (E_H) was higher than that in low temperature ranges (E_L) (Fig. 4 Panels D-F). The data on the energies of activation and phase transition temperature are given in Table 3. In control group the values of E_H and E_L were about 40 and 70 KJ/mole with phase transition at around 16.5 °C. At early s well as late diabetic states the value of E_H increased. At early state of diabetes E_L was unchanged whereas substantial reduction (63 % decrease) was noted at late stage. Insulin treatment had restorative effect only on E_H at early stage of diabetes. At late stage effects on E_H and E_L persisted even after insulin treatment. At early stage of diabetes phase transition temperature (T_t) was unchanged while at late stage it increased; insulin treatment was ineffective (Table 3).

In view of the observed changes in the substrate and temperature kinetics properties of ATPase and G6Pase, the effects of insulin status on lipid/phospholipid profiles of the microsomal membrane were examined. The results are given in Tables 4-6. In early diabetic stage the total phospholipid (TPL) content of the microsomal membranes decreased by 19 % while the cholesterol (CHL) content increased by 50 %. Insulin treatment restored the TPL content whereas the CHL content increased further. A similar 21 % decrease in the TPL content persisted at the late stage of diabetes. By contrast, the CHL content increased substantially by 2.4 fold. Insulin treatment had no effect on TPL content but marginally lowered the CHL content. The TPL/CHL (mole : mole) ratios

Table 3. Effect of alloxan-diabetes and insulin treatment on Arrhenius kinetics properties of Na^+, K^+- ATPase in rat brain microsomes

		Energy of activation (KJ/mole)		Phase transition temperature Tt (°C)
Group	Treatment	E_H	E_L	T_t
One week	Control	40.28 ± 1.41	70.60 ± 4.09	16.88 ± 0.18
	Diabetic	50.04 ± 1.08[b]	77.07 ± 2.30	15.69 ± 0.61
	Diabetic + Insulin	43.15 ± 0.81[Ψ]	77.84 ± 2.15	17.18 ± 0.58
One month	Control	42.35 ± 1.23	71.86 ± 1.36	16.23 ± 0.30
	Diabetic	48.31 ± 1.01[b]	26.84 ± 0.96[b]	20.10 ± 1.00[a]
	Diabetic + Insulin	53.29 ± 0.61[b,§]	33.31 ± 0.45[b,§]	19.89 ± 0.80[Ψ]

The experimental details are given in the text. The results are given as mean ± SEM of 6-8 independent experiments.

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

Ψ, $p<0.002$ and §, $p<0.001$ compared to the corresponding diabetic.

Table 4. Effects of alloxan diabetes on total phospholipids (TPL), cholesterol (CHL) and TPL/CHL ratio in rat brain microsomes

Groups	Treatment	TPL (μg/mg protein)	CHL (μg/mg protein)	TPL/CHL (mole:mole)
One week	Control	683.1 ± 11.4	213.3 ± 7.42	1.62 ± 0.07
	Diabetic	552.2 ± 9.84[a]	321.0 ± 7.85[a]	0.87 ± 0.03[a]
	Diabetic + Insulin	669.0 ± 10.3[Ψ]	443.3 ± 10.6[a,Ψ]	0.76 ± 0.03[a,§]
One month	Control	668.2 ± 11.8	208.4 ± 8.44	1.63 ± 0.08
	Diabetic	526.5 ± 21.7[a]	492.5 ± 18.1[a]	0.54 ± 0.02[a]
	Diabetic + Insulin	503.1 ± 11.2[a]	456.1 ± 10.5[a]	0.55 ± 0.02[a]

The results are given as mean ± SEM of the number of independent observations indicated in parentheses.

a, $p<0.001$ compared to the corresponding control.
§, $p<0.05$ and Ψ, $p< 0.001$ compared to the corresponding diabetic.

Table 5. Effects of alloxan diabetes and insulin treatment on phospholipid composition in rat brain microsomes.

Phospholipid Class	Composition (% of total)					
	One week			One month		
	Control	Diabetic	Diabetic + Insulin	Control	Diabetic	Diabetic + Insulin
Lyso	3.84 ± 0.09	3.77 ± 0.13	2.45 ± 0.18$^{b,\psi}$	3.64 ± 0.10	3.49 ± 0.12	1.16 ± 0.06$^{b,\psi}$
SPM	8.58 ± 0.12	10.91 ± 0.34^{b}	8.12 ± 0.31$^{\psi}$	8.73 ± 0.10	8.86 ± 0.19	6.86 ± 0.23$^{b,\psi}$
PC	45.31 ± 0.56	44.52 ± 0.59	46.88 ± 0.56§	44.19 ± 0.88	37.44 ± 0.64^{b}	50.77 ± 0.88$^{b,\psi}$
PI	5.05 ± 0.16	4.14 ± 0.20^{a}	2.66 ± 0.15$^{b,\psi}$	4.65 ± 0.31	6.29 ± 0.34^{a}	1.08 ± 0.04$^{b,\psi}$
PS	5.82 ± 0.10	4.38 ± 0.22^{b}	2.75 ± 0.18$^{b,\psi}$	5.33 ± 0.19	8.42 ± 0.66^{b}	3.72 ± 0.16$^{b,\psi}$
PE	27.50 ± 0.40	28.37 ± 0.86	34.34 ± 0.63$^{b,\psi}$	28.46 ± 0.41	31.42 ± 0.66^{a}	34.81 ± 0.83^{b,§}
PA	3.84 ± 0.21	3.85 ± 0.20	2.74 ± 0.20^{a,¶}	4.02 ± 0.11	4.11 ± 0.19	1.54 ± 0.04$^{b,\psi}$

The results are given as mean ± SEM of the 6-8 independent observations.

Lyso: Lysophospholipid; SPM: sphinghomyelin; PC: phosphatidylcholine; PI: phosphatidylinositol; PS: phosphatidylserine; PE: phosphatidylethanolamine; PA: phosphatidicacid

a, $p< 0.01$ and b, $p<0.001$ compared to the corresponding control.
§, $p< 0.02$; ¶, $p<0.01$ and, ψ $p<0.001$ compared to the corresponding diabetic.

Table 6. Effects of alloxan-diabetes and insulin treatment on phospholipid content in rat brain microsomes

Phospholipid Class	Content, μg / mg protein					
	One week			One month		
	Control	Diabetic	Diabetic + Insulin	Control	Diabetic	Diabetic + Insulin
Lyso	26.11 ± 0.63	20.90 ± 0.98[d]	15.35 ± 1.25[d,ψ]	26.67 ± 0.70	18.58 ± 1.09[d]	5.85 ± 0.35[c,‡]
SPM	58.41 ± 1.16	60.67 ± 2.67	52.43 ± 2.36[b,§]	57.70 ± 1.42	46.75 ± 1.69[d]	34.51 ± 1.47[d,‡]
PC	308.88 ± 4.52	245.90 ± 6.26[d]	312.96 ± 6.40[‡]	308.43 ± 5.99	197.78 ± 7.22[d]	255.73 ± 6.05[d,‡]
PI	34.28 ± 0.98	22.76 ± 0.94[d]	18.41 ± 1.14[b,¶]	34.66 ± 1.23	33.29 ± 2.13	5.40 ± 0.22[d,‡]
PS	39.56 ± 0.70	24.10 ± 1.15[d]	17.78 ± 1.55[d,ψ]	40.02 ± 0.77	44.46 ± 1.73[a]	18.72 ± 0.94[d,‡]
PE	187.40 ± 4.04	156.73 ± 4.76[d]	233.13 ± 5.54[d,‡]	185.40 ± 5.18	166.48 ± 7.38	175.15 ± 3.75
PA	26.06 ± 1.39	21.12 ± 0.93[b]	18.88 ± 1.75[d]	26.42 ± 1.70	21.57 ± 0.98[a]	7.75 ± 0.27[d,‡]

The experimental details are given in the text. The results are given as mean ± SEM of 6-8 independent experiments.

a, $p< 0.02$; b, $p< 0.01$; c, $p<0.002$ and d, $p<0.001$ compared to the corresponding control.
§, $p< 0.05$; ¶, $p<0.02$; ψ, $p<0.01$ and ‡, $p<0.001$ compared to the corresponding diabetic.

decreased progressively in the diabetic groups; insulin treatment had no corrective effect (Table 4).

The data on phospholipid composition of microsomal membranes are given in Table 5. Early diabetic state resulted in increase in the sphingomyelin (SPM) component with simultaneous decrease in the phosphatidylinositol (PI) and phosphatidylserine (PS) components. Following insulin treatment, SPM became comparable to control whereas PI, PS components decreased further. Insulin treatment also resulted in lowering the proportion of lysophospholipids (Lyso), and phosphatidic acid (PA) components. Under these conditions, the phosphatidylethanolamine (PE) component increased significantly with only marginal changes in phosphatidylcholine (PC). In one month diabetic animals the PI, PS and PE components increased and the PC component decreased. Insulin treatment caused significant decrease in Lyso, SPM, PI, PS and PA whereas PC and PE registered significant increase (Table 5). The computed contents of the individual phospholipid classes were generally consistent with the compositional changes (Table 6).

Changes in the levels of cholesterol and altered phospholipid composition and contents could alter the fluidity of the membrane. This was ascertained by measuring the fluidity of the membranes. The membrane fluidity decreased in diabetic animals; insulin treatment significantly fluidized the membrane in one week diabetic animals but had no appreciable effect in the one month diabetic animals (Table 7).

Discussion

Data of present study show that insulin status significantly altered the kinetic properties of the brain microsomal ATPase. Thus, although at early stage of diabetes the enzyme

Table 7. Effects of alloxan-diabetes and insulin treatment on fluidity parameters of rat brain microsomes

Groups	Treatment	Fluidity Parameters			
		Fluorescence Polarization, p	Fluorescence anisotropy, r	Limited hindered anisotropy, rα	Order parameter, S
One week	Control	0.228 ± 0.009	0.165 ± 0.008	0.119 ± 0.007	0.546 ± 0.025
	Diabetic	0.248 ± 0.008	0.180 ± 0.009	0.140 ± 0.007[a]	0.592 ± 0.026
	Diabetic + Insulin	0.149 ± 0.006[d,§]	0.105 ± 0.005[d,§]	0.039 ± 0.002[d,§]	0.314 ± 0.010[d,§]
One month	Control	0.232 ± 0.013	0.168 ± 0.010	0.124 ± 0.008	0.556 ± 0.024
	Diabetic	0.281 ± 0.008[c]	0.207 ± 0.010[b]	0.176 ± 0.008[d]	0.663 ± 0.031[a]
	Diabetic + Insulin	0.274 ± 0.007[b]	0.201 ± 0.009[a]	0.168 ± 0.009[d,§]	0.648 ± 0.016[d,§]

The experimental details are given in the text. The results are given as mean ± SEM of 6-8 independent experiments in each group.

a, $p< 0.05$; b, $p< 0.02$; c, $p<0.01$ and d, $p<0.001$ compared to the corresponding control.
§, $p< 0.001$ compared to the corresponding diabetic.

activity was unchanged at late stage it decreased significantly (Table 1). Also, the substrate kinetics analysis revealed that the Km of component I increased significantly. The increased Km is suggestive of increased demand for ATP for the normal function of the microsomal enzyme. However, earlier studies from our laboratory showed that in streptozotocin-diabetes, the overall oxidative capacity of the brain mitochondria decreased significantly at the late stage of the disease (3).

It can also be noted that the apparent efficiency index (Chapter 8 of the Thesis) decreased in diabetic state progressively for the component I. At early stage insulin treatment failed in correcting the apparent efficiency index whereas at late stage insulin treatment checked the apparent efficiency index. For component II similar picture was noted in one week experimental group. However, at late stage even after insulin treatment the apparent efficiency index remained somewhat low (data not shown but can be calculated easily from Vmax and Km value in Table 2).

Studies on temperature kinetics showed that the diabetic state resulted in significant increase in the energy of activation in high temperature ranges which is suggestive of decreased efficiency of the enzyme. However, at late stage the energy of activation in low temperature range decreased to less than half. This may probably reflect a compensatory mechanism for efficient functioning of the enzyme in spite of the increase in Km.
As pointed out earlier, regulation of Na^+, K^+-ATPase is a complex process and several factors known to regulate this process (4-8). Data of the present study on phospholipid composition suggest that early diabetic stage lowered the acidic phospholipids PI and PS

whereas at late stage PI and PS increased significantly. At late stage, the PC component decreased whereas PE increased. Insulin treatment had differential effects on the phospholipid classes.

Regression analysis across the groups between kinetics properties of Na^+, K^+-ATPase and lipid/phospholipid classes revealed that, the acidic phospholipid PS and PI did not seem to have any regulatory role on the substrate kinetic properties of ATPase except that PS correlated negatively with Vmax of component II. This is in contrast with the known requirement of acidic phospholipids for the activity of the plasma membrane Na^+, K^+-ATPase (4, 5). Regression analysis also revealed that CHL and PE are positive regulators of Km component I and II respectively. SPM seemed to be the positive regulator of Vmax of component I.

TPL correlated positively the energy of activation in low temperature range while opposite regulatory role was seen for the energy of activation in high temperature range. Also, TPL negatively correlated with phase transition temperature. CHL had opposite effects on these three parameters. It may hence be suggested that also in the brain, phospholipid requirement of the plasma membrane and microsomal enzyme may be quite different (Table 8).

Table 8. Correlation of kinetic parameters of brain microsomal Na^+, K^+-ATPase with membrane lipid/phospholipid composition

Parameter	Correlation with Phospholipid Class	
	Positive	Negative
Substrate kinetics		
Km_1	CHL (+ 0.599)	—
Km_2	PE (+ 0.631)	—
$Vmax_1$	SPM (+ 0.658)	—
$Vmax_2$	TPL (+ 0.745)	PS (- 0.717)
Temperature Kinetics		
E_H	CHL (+ 0.628)	TPL (+ 0.961)
E_L	TPL (+ 0.754)	CHL (- 0.651)
T_t	CHL (+ 0.763)	TPL (- 0.763)

The experimental details are given in the text.

Values given in the parentheses indicate r, regression coefficient which is based on 6-8 independent experiments in each group.

Summary

The Na^+, K^+-ATPase activity decreased in diabetic groups. Insulin treatment in one week diabetic animals increased the values beyond control whereas in one month diabetic group partial restoration was noted.

The brain microsomal Na^+, K^+-ATPase also displayed two kinetic components in all the experimental conditions.

The Km of component I increased whereas that of component II decreased in one week diabetic group; insulin treatment had no effect on the Km of component I but resulted in elevating the Km of component II. In one month diabetic group Km of component I increased which was corrected following insulin treatment.

In early diabetic stage, Vmax of component I increased whereas that of component II decreased. Insulin treatment resulted in significant increase in the Vmax of component II. At late stage of diabetes Vmax of component II decreased which was marginally restored by insulin treatment.

The Hill plot analysis suggested that up to 0.7 mM ATP concentration one ATP molecule was bound while beyond this concentration two ATP molecules were bound to the enzyme under all the experimental conditions.

Temperature kinetics studies revealed that late diabetic stage as well as insulin treatment reversed the Arrhenus pattern. In diabetic animals E_H increased which was restored by insulin treatment only at early stage. E_L decreased significantly at late stage of diabetes and insulin treatment was ineffective in correcting the same. T_t increased significantly in

one month diabetic animals and insulin treatment was again ineffective in restoring the values to normality.

The lipid/phospholipids analysis showed that in early diabetic stage the TPL content decreased whereas CHL content increased. Insulin treatment restored the TPL content while CHL content increased further. At the late stage of diabetes decrease in TPL content, by contrast, the CHL content increased substantially. Insulin treatment had no effect on TPL content but marginally lowered the CHL content.

The membrane fluidity decreased in diabetic animals and treatment with insulin significantly fluidized the membrane in one week diabetic animals; no appreciable effect was seen in the one month diabetic animals.

In one week diabetic group SPM increased and PI and PS decreased whereas in one month diabetic animals PI, PS and PE increased while PC decreased. Insulin treatment in one week diabetic animals decreased the proportion of Lyso, PI, PS and PA components while PE increased. In one month diabetic animals, insulin treatment caused significant decrease in Lyso, SPM, PI, PS and PA; PC and PE increased.

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