Chapter 4

Insulin-Status-Dependant Modulation of FoF_1 ATPase Activity in Rat Brain Mitochondria

Introduction

Diabetic neuropathy is a common and devastating complication of type 1 and type 2 diabetes mellitus (1-4). In the diabetic patients the peripheral and autonomic nervous system is affected initially (1-4) and with time the central nervous system (CNS) also gets affected (5). The sensory neurons are the primary target and their involvement accounts for prominent sensory loss and pain in the diabetic patients (6, 7). The factors responsible include excessive polyol flux, advanced glycation end products (AGEs), impaired essential fatty acid metabolism and oxidative stress (1, 8).

In our earlier studies we have noted that, streptozotocin-diabetes and subsequent insulin treatment significantly altered the oxidative energy metabolism in the rat brain mitochondria (9). As noted earlier (Chapter 2 and 3 of the Thesis) we observed that alloxan diabetes and treatment with insulin significantly influenced the kinetic properties of liver and kidney mitochondrial ATPase and lipid/phospholipid make-up in a tissue-specific manner.

It was therefore of interest to find out if the insulin status also affects the brain mitochondrial ATPase as well as lipid/phospholipid profiles. Hence the early and late effects of alloxan-diabetes and subsequent insulin treatment on kinetic properties of FoF_1 ATPase and lipid/phospholipid profile in rat brain mitochondria were examined. The results are summarized in this Chapter.

Materials and methods

Chemicals

Details of chemicals used, procedures of induction of diabetes, insulin treatment and isolation of mitochondria are as described in Chapter 2 of the Thesis.

ATPase assay

ATPase activity in mitochondria was measured under optimized conditions as detailed below. The assay medium (0.1 ml) consisted of 250 mM sucrose, 10 mM KCl, 0.2 mM EDTA, 2 mM $MgCl_2$ and 50 μM DNP. After incubating 35-50 μg mitochondrial protein in the assay medium at 37 °C for 1 min, the reaction was initiated by the addition of ATP at the final concentration of 2 mM. The reaction was carried out for 10 min and then terminated by adding 1.1 ml 5 % (w/v) trichloroacetic acid (TCA) solution. The tubes were kept on ice for 15 min and then centrifuged for 10 min at 3000 rpm. Known aliquots of supernatant were used for estimation of released in organic phosphorus.

Analytical procedures

Detailed o the procedure used for the extraction of mitochondrial lipids/phospholipids, estimation of cholesterol, determination of phospholipid profile, membrane fluidity and data analysis are as described in Chapter 2 of the Thesis.

Results

The brain weight was unchanged at the early stage of diabetes whereas in one month diabetic group the brain weight decreased by 17%; insulin treatment had only marginal

restorative effect (Table 1). These results are consistent with our previously reported observations (9).

Results of diabetes parameters i.e. polyuria, glucosuria are as described in the Chapter 2 of the Thesis.

In the initial experiments, effect of alloxan-diabetes and subsequent insulin treatment on ATPase activity under different conditions was evaluated (Table 2). As is evident, addition of Mg^{2+} stimulated the enzyme activity maximally whereas stimulation with DNP is not of that magnitude. In presence of both Mg^{2+} and DNP the activity was intermediate. In one week diabetic group the enzyme activity was unchanged. Insulin treatment resulted in hyper-stimulation. At the later stage of diabetes the enzyme activity decreased. Upon insulin treatment the activity increased beyond control levels (Table 3).

Measurements at 25 and 37 °C reveled that in one week diabetic animals the activity did not change whereas in one month diabetic animals the enzyme activity decreased by 12 and 15 % at the two temperatures, respectively. Insulin treatment resulted in hyper-stimulation of the enzyme activity in both diabetic groups. Activity ratio did not change in diabetic animals whereas insulin treatment lowered the activity ratio (Table 3).

Since the diabetic state and insulin treatment significantly influenced the enzyme activity, in the next sets of experiments the kinetic behavior of the enzyme as a response to change in the substrate i.e. ATP concentration and change in temperature was examined.

Table 1. Effect of alloxan diabetes and insulin treatment on body and brain weight of the rats

Treatment Period	Group	Final body weight, g	Brain weight	
			g	% of body weight
One week	Control	253.0 ± 3.6	1.61 ± 0.04	0.64 ± 0.020
	Diabetic	213.0 ± 5.9[c]	1.64 ± 0.03	0.77 ± 0.023[b]
	Diabetic + Insulin	238.0 ± 6.7[§]	1.69 ± 0.03	0.71 ± 0.035
One month	Control	274.7 ± 4.7	1.70 ± 0.05	0.62 ± 0.024
	Diabetic	187.9 ± 10.02[c]	1.41 ± 0.05[b]	0.75 ± 0.038[a]
	Diabetic + Insulin	288.5 ± 7.2[Ψ]	1.59 ± 0.03[¶]	0.55 ± 0.032[¶]

The experimental details are given in the text. The results are given as mean ± S. E. M. of 6-8 independent observations.

a, $p<0.02$; b, $p< 0.002$ and c, $p< 0.001$ compared with control values.
§, $p<0.02$; ¶, $p< 0.002$ and Ψ, $p< 0.001$ compared with corresponding diabetic values.

Table 2. Effect of alloxan-diabetes and insulin treatment on FoF_1 ATPase activity in rat brain mitochondria

Group	Treatment	Activity, μmole of Pi liberated / hr / mg protein			
		Basal	+ Mg	+DNP	+Mg, + DNP
One week	Control	0.24 ± 0.02	3.08 ± 0.18	1.03 ± 0.08	2.99 ± 0.19
	Diabetic	0.21 ± 0.01	2.84 ± 0.13	0.92 ± 0.07	2.78 ± 0.16
	Diabetic + Insulin	0.35 ± 0.02[b,§]	6.41 ± 0.43[e,§]	1.24 ± 0.08	5.33 ± 0.34[e,§]
One Month	Control	0.26 ± 0.02	3.15 ± 0.20	1.00 ± 0.05	3.07 ± 0.22
	Diabetic	0.17 ± 0.01[d]	2.58 ± 0.14[a]	0.79 ± 0.04[c]	2.14 ± 0.13[c]
	Diabetic + Insulin	0.31 ± 0.02[§]	4.21 ± 0.22[c,§]	1.15 ± 0.07[§]	3.91 ± 0.16[b,§]

The experimental details are given in the text. The results are given as mean ± S. E. M. of 6-8 independent observations.

a, $p<0.05$; b, $p< 0.02$; c, $p<0.01$; d, $p< 0.002$ and e, $p< 0.001$ compared with control values.
§, $p< 0.001$ compared with corresponding diabetic values.

Table 3. Effect of alloxan diabetes and insulin treatment on FoF_1 ATPase activity in rat brain mitochondria

Group	Treatment	Activity (μmole of Pi liberated / hr / mg protein)		Activity Ratio
		25 °C	37 °C	
One week	Control	1.22 ± 0.05	2.88 ± 0.19	2.36 ± 0.12
	Diabetic	1.13 ± 0.07	2.54 ± 0.11	2.25 ± 0.09
	Diabetic + Insulin	2.90 ± 0.10[c,§]	5.85 ± 0.16[c,§]	2.02 ± 0.08
One month	Control	1.21 ± 0.04	3.20 ± 0.15	2.64 ± 0.10
	Diabetic	1.07 ± 0.03[b]	2.73 ± 0.11[a]	2.55 ± 0.08
	Diabetic + Insulin	2.17 ± 0.17[c,§]	3.93 ± 0.19[b,§]	1.81 ± 0.09

The experimental details are given in the text.

Activity ratio was calculated as: activity at 37 °C/activity at °C

The results are given as mean ± S. E. M. of 6-8 independent observations.

a, $p<0.05$; b, $p< 0.02$ and c, $p<0.001$ compared with control values.
§, $p< 0.001$ compared with corresponding diabetic values.

The typical substrate saturation curves and corresponding Eadie-Hofstee plots are shown in Fig. 1 and 2 Panels A, B, C and D, E, F. As can be noted, the substrate saturation curve for controls was sigmoidal (Fig. 1 and 2 Panels A). The pattern remained the same for one week diabetic and insulin treated one week diabetic groups (Fig. 1 Panels B and C) whereas typical Michaelis-Menten pattern was observed in one month diabetic and one month insulin treated diabetic groups (Fig 2 Panel B and C). These differences were confirmed when the data were transformed in Eadie-Hofstee plots (Fig. 1 and 2 Panels D, E, F). As can be seen from the corresponding Eadie-Hofstee plots the ATPase activity in one month diabetic and one month insulin treated diabetic animals resolved in two kinetic components whereas all other groups displayed allosteric behavior.

The values of Km and Vmax for one month diabetic and one month insulin treated diabetic groups are given in Table 4. Since the pattern was allosteric in other groups (Fig. 1 Panels A, B, C and Fig. 2 Panel A), it is not possible to calculate Km and Vmax values.

Typical Hill plots are shown in Fig. 1 and 2, Panels G, H, I and data is given in Table 5. It can be noted that, at the late diabetic stage Hill coefficients n_1 and n_2 decreased. Insulin treatment had no restorative effects. It is also seen that up to 0.35 mM ATP concentration one ATP molecule was bound while beyond this concentration apparently two ATP molecules were bound to the enzyme under all the experimental conditions.

In the next set of experiments the temperature dependence of the enzyme activity was examined. The typical temperature curve (Fig. 3 and 4, Panels A, B, C) and corresponding Arrhenius plots (Fig. 3 and 4, Panels A, B, C) are shown. The difference in the temperature curves are self evident (Fig. 1 and 2, Panels A, B, C). As evident, the

Figure 1

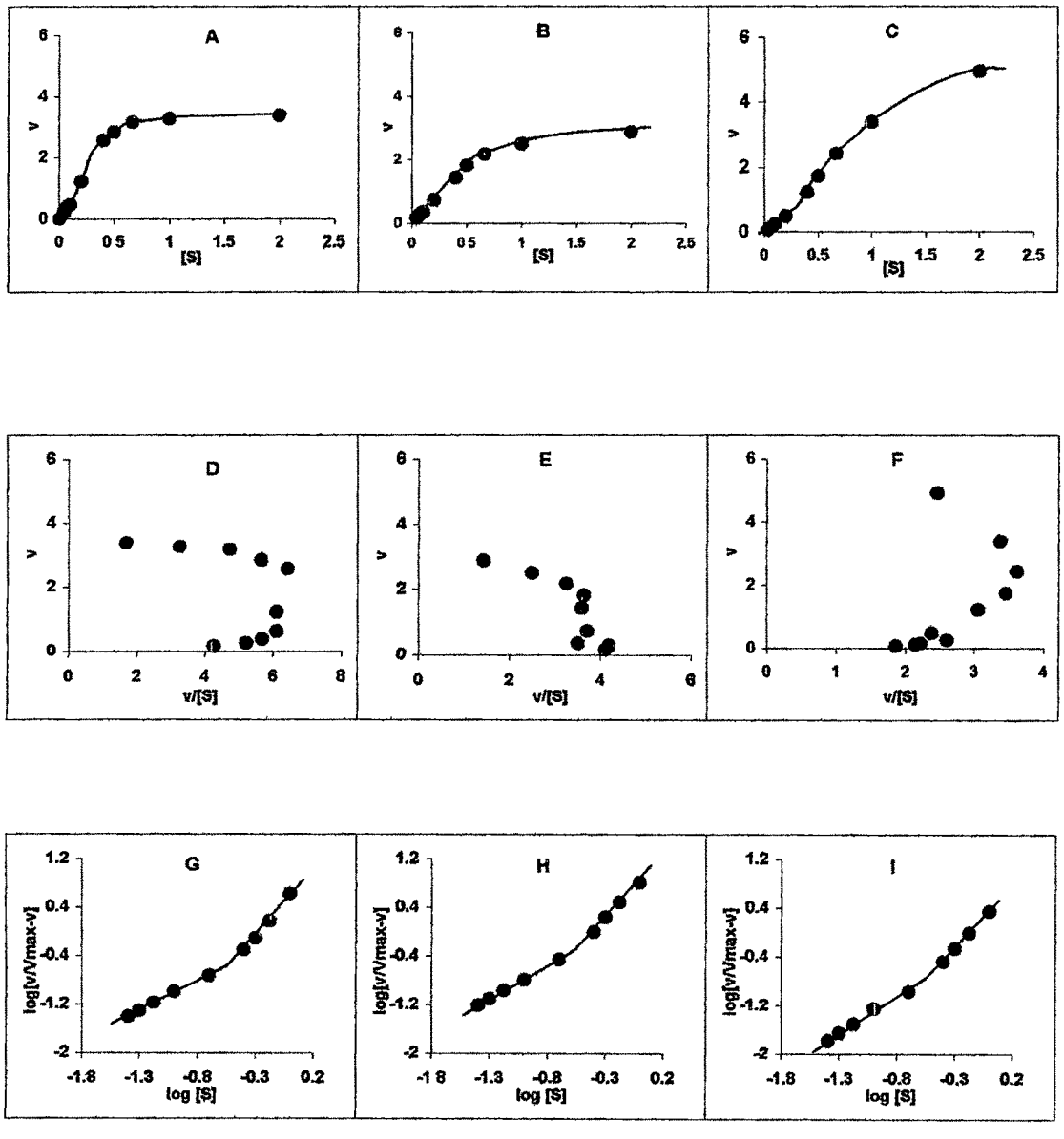

Figure 2

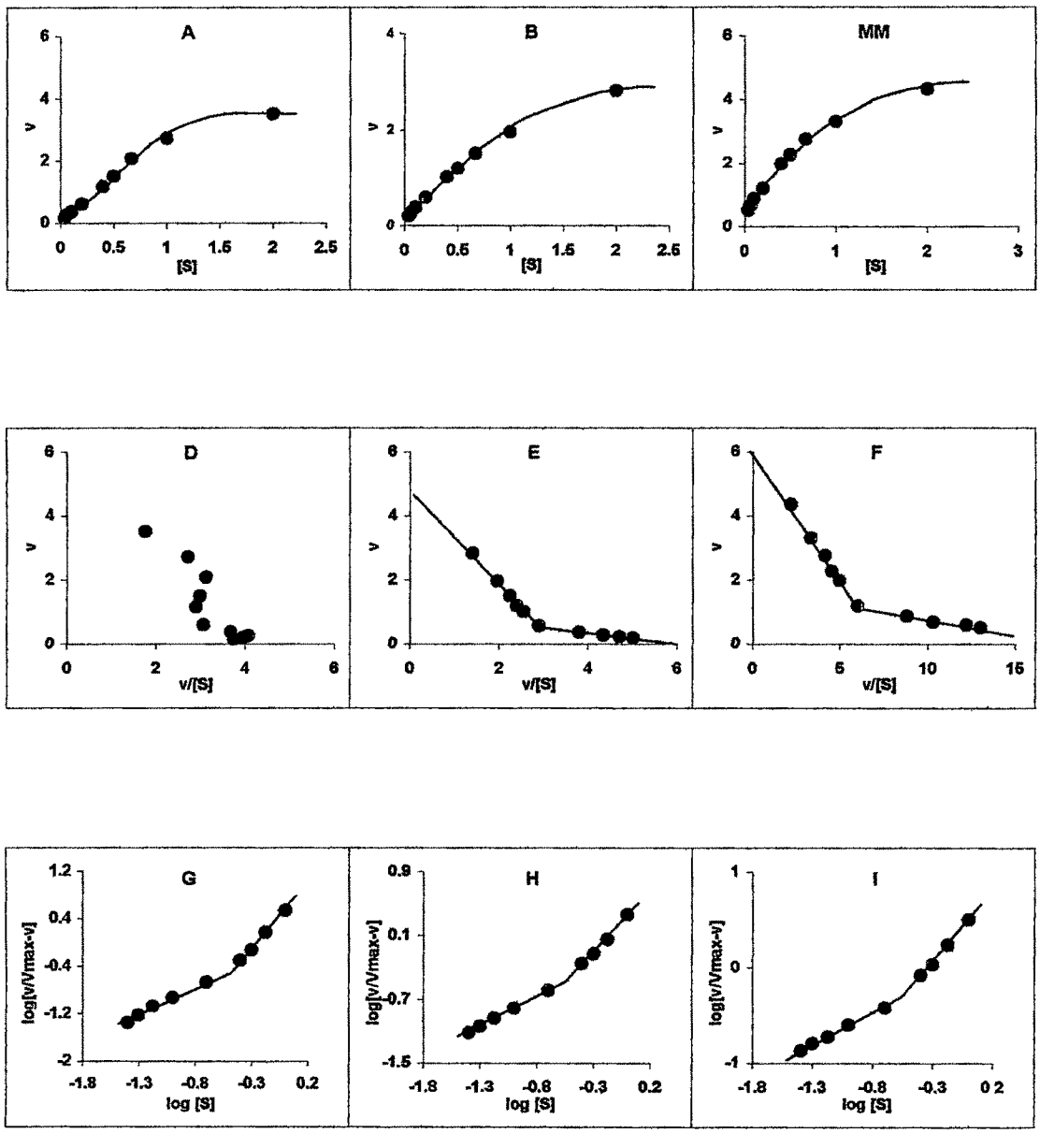

Figure 1. Typical substrate saturation curve (Panels A, B, C), corresponding Eadie-Hofstee (Panels D, E, F) and Hill (Panels G, H, I) plots for FoF_1 ATPase from brain mitochondria for one week group. In substrate saturation curve the enzyme activity v on abscissa is plotted versus [S] on ordinate v 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 FoF_1 ATPase from brain mitochondria 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 4. Effect of alloxan-diabetes and insulin treatment on substrate kinetics properties in rat brain mitochondrial ATPase

Group	Treatment	Component I		Component II	
		Km	Vmax	Km	Vmax
One week	Control	—	—	—	—
	Diabetic	—	—	—	—
	Diabetic + Insulin	—	—	—	—
One month	Control	—	—	—	—
	Diabetic	0.23 ± 0.004	0.99 ± 0.014	1.88 ± 0.074	4.82 ± 0.128
	Diabetic + Insulin	0.08 ± 0.005§	1.77 ± 0.079§	0.95 ± 0.054§	6.74 ± 0.340§

The Km (mM) and Vmax (μmole of Pi liberated / hr / mg protein) values were calculated as described in the text. The experiments were carried out at 37 °C.

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.

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

Table 5. Effect of alloxan-diabetes and insulin treatment on Hill plot analysis on rat brain mitochondrial FoF_1 ATPase

Group	Treatment	Hill coefficient		Transition concentration point, mM
		n_1	n_2	
One week	Control	1.03 ± 0.07	2.07 ± 0.09	0.312 ± 0.020
	Diabetic	1.14 ± 0.02	2.38 ± 0.16	0.359 ± 0.023
	Diabetic + Insulin	1.17 ± 0.03	1.86 ± 0.07	0.285 ± 0.019
One month	Control	1.06 ± 0.05	2.05 ± 0.09	0.304 ± 0.020
	Diabetic	0.81 ± 0.02[a]	1.53 ± 0.02[a]	0.288 ± 0.015
	Diabetic + Insulin	0.68 ± 0.05[a,§]	1.53 ± 0.05[a]	0.279 ± 0.019

The experimental details are given in the text. The results are given as mean ± S. E. M. of 6-8 independent observations.

a, $p < 0.001$ compared with control values.
§, $p < 0.05$ compared with corresponding diabetic values.

optimum temperature for the control groups was beyond 53 °C which decrease to 45 °C in both the diabetic as well as insulin treated diabetic groups (Fig. 3 and 4, Panels A, B, C). In addition to this, the Arrhenius plots for the controls was chair shaped with two phase transition temperatures around 19 and 34 °C. One week and one month diabetic groups displayed biphasic Arrhenius plots with single phase transition temperature 17 and 13 °C respectively which increased upon insulin treatment at late stage (Fig. 3 and 4, Panels D, E, F; Table 5). Other researchers have also reported chair shaped Arrhenius plots for mitochondrial ATPase and cytochrome oxidase (10, 11). In control animals, the energy of activation in intermediate temperature ranges (E_I) was highest (about 74 KJ/mole) whereas that of in low and high temperature ranges (E_L and E_H respectively) the values were much lower and are almost comparable with each other (Table 5). In both the diabetic groups the energy of activation in low temperature ranges (E_L) did not changes whereas in high temperature ranges the energy of activation (E_H) increased significantly (Table 5). Insulin treatment partially restored the E_H in one week diabetic animals while no restorative effect was seen in one month diabetic animals (Table 5).

Since insulin status considerably altered the substrate and temperature kinetic properties of ATPase; in the next set of experiments the effects of alloxan-diabetes and insulin treatment on lipid/phospholipid make-up of the brain mitochondria was evaluated. The data in Table 6 show that the TPL and CHL contents decreased by 34 and 19 % respectively in one week diabetic animals. Insulin treatment had no effect on CHL content but partially restored the TPL content. In one month diabetic animals the TPL and CHL contents were unchanged. Insulin treatment lowered the TPL and CHL contents by 15 and 36 %. The TPL/CHL (mole : mole) ratios decreased in the one week diabetic

Figure 3

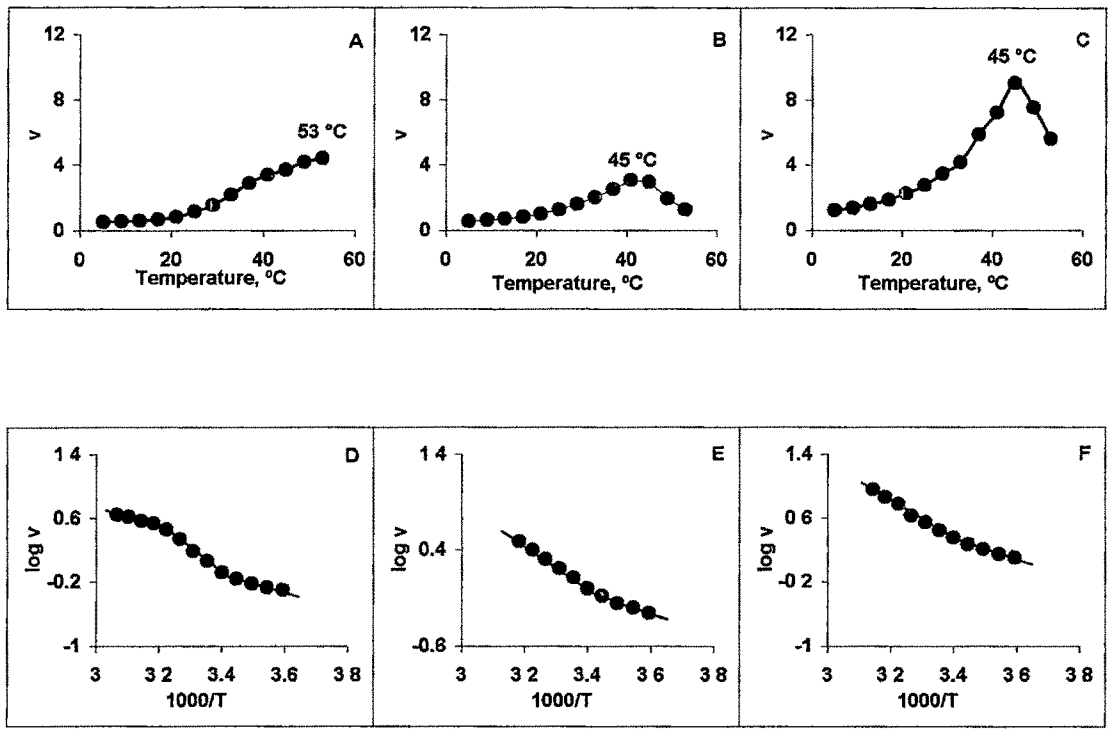

Figure 4

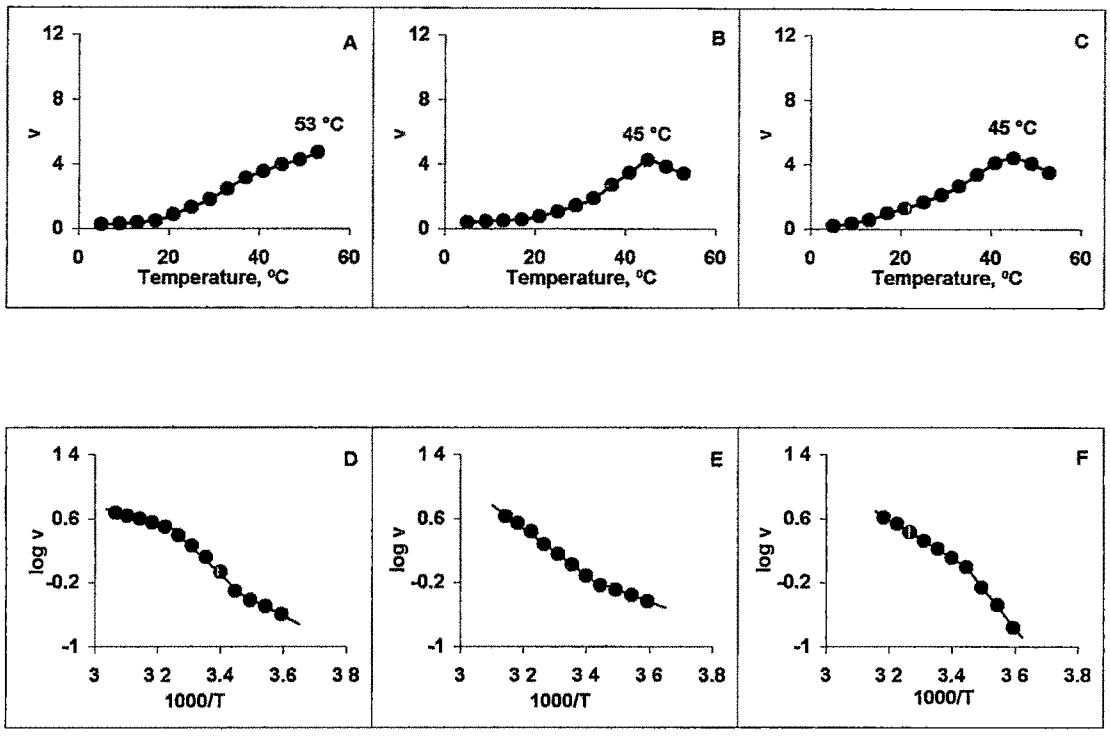

Figure 3. Typical plots depicting dependence of enzyme activity on the temperature and corresponding Arrhenius plots for one week groups. In temperature curves, enzyme activity v on abscissa is plotted verses temperature (°C) on ordinate. A, B and C represent to the control, diabetic and insulin-treated 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 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.

Table 6. Effect of alloxan-diabetes and insulin treatment on Arrhenius kinetics property in rat brain mitochondrial ATPase

		Energy of activation KJ/mole			Phase transition temperature Tt (°C)	
Group	Treatment	E_L	E_I	E_H	Tt_1	Tt_2
One week	Control	29.33 ± 2.21	74.05 ± 2.86	24.61 ± 1.27	19.42 ± 0.85	34.74 ± 1.26
	Diabetic	30.19 ± 1.40	—	64.67 ± 1.73[a]	17.59 ± 0.63	—
	Diabetic + Insulin	28.18 ± 1.60	—	43.71 ± 0.77[a,§]	20.39 ± 0.97	—
One month	Control	28.11 ± 1.47	73.84 ± 2.54	24.03 ± 1.68	19.15 ± 0.81	33.98 ± 0.72
	Diabetic	26.97 ± 2.35	—	61.31 ± 0.98[a]	13.01 ± 0.83[a]	—
	Diabetic + Insulin	59.06 ± 3.48[a,§]	—	25.03 ± 1.18[§]	21.86 ± 1.01[§]	—

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

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

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

Table 7. Effects of alloxan diabetes on total phospholipids (TPL), cholesterol (CHL), TPL/CHL ratio in rat brain mitochondria

Groups	Treatment	TPL (μg/mg protein)	CHL (μg/mg protein)	TPL/CHL (mole:mole)
One week	Control	478.6 ± 5.56	446.1 ± 6.43	0.54 ± 0.01
	Diabetic	318.2 ± 10.4[b]	359.0 ± 16.0[b]	0.45 ± 0.02[a]
	Diabetic + Insulin	442.8 ± 10.4[b,Ψ]	364.3 ± 5.26[b]	0.61 ± 0.01[Ψ]
One month	Control	475.7 ± 5.38	461.0 ± 5.47	0.52 ± 0.01
	Diabetic	451.1 ± 13.8	457.9 ± 18.5	0.49 ± 0.01
	Diabetic + Insulin	403.9 ± 8.34[b,§]	294.4 ± 5.40[b,Ψ]	0.68 ± 0.01[b,Ψ]

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

a, $p<0.002$ and b, $p<0.001$ compared to the corresponding control.

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

group; insulin treatment elevated the TPL/CHL (mole : mole) ratios in both the groups.

The data on phospholipid composition are given in Table 8. In one week diabetic animals the phospholipid composition was practically unchanged except for a significant reduction in lysophospholipids (Lyso) and a tendency towards decrease in diphosphatidylglycerol (DPG) component. Insulin treatment lowered the Lyso and phosphatidylserin (PS) composition while DPG increased significantly with marginal increase in sphingomyelin (SPM) compared to the control group. In one month diabetic animals also the phospholipid composition was practically unchanged except for a small reduction in Phosphatidylethanolamine (PE). Insulin treatment caused significant reduction in phosphatidylinositol (PI) and PS components and almost 2 fold increases in DPG (Table 9). The data on phospholipid content are given in Table 8 and are consistent with the composition data.

The membrane fluidity increased in one week diabetic animals and insulin treatment further fluidized the membrane. In one month diabetic animals the membrane fluidity increased marginally and insulin treatment restored it to normality (Table 10).

Discussion

From the data presented, it can be seen that at early stage of diabetes the ATPase activity did not change whereas it decreased significantly at late stage (Table 3). Similarly from the substrate kinetics data it is evident that the early diabetic state had no effects whereas the substrate kinetic properties of the enzyme the late diabetic state (Fig. 1 and 2, Table 4 and 5). Our earlier reports suggest that in one month diabetic animals the oxidative

Table 8. Effects of alloxan diabetes and insulin treatment on phospholipid composition in rat brain mitochondria.

Phospholipid Class	Composition (% of total)					
	One week			One month		
	Control	Diabetic	Diabetic + Insulin	Control	Diabetic	Diabetic + Insulin
Lyso	3.40 ± 0.17	1.64 ± 0.11[c]	2.28 ± 0.22[b,§]	3.62 ± 0.23	3.31 ± 0.22	3.49 ± 0.10
SPM	7.44 ± 0.19	7.40 ± 0.26	8.35 ± 0.19[a,¶]	7.38 ± 0.34	8.23 ± 0.31	8.64 ± 0.20[a]
PC	39.32 ± 0.49	41.30 ± 0.96	40.68 ± 0.60	41.82 ± 0.99	41.88 ± 0.38	41.71 ± 0.33
PI	3.64 ± 0.12	3.56 ± 0.17	3.58 ± 0.03	3.21 ± 0.10	3.32 ± 0.21	2.29 ± 0.09[c,ψ]
PS	4.51 ± 0.15	4.57 ± 0.24	3.47 ± 0.22[a,¶]	4.31 ± 0.17	4.80 ± 0.29	1.26 ± 0.05[c,ψ]
PE	37.87 ± 0.47	38.30 ± 0.47	36.71 ± 0.55	37.67 ± 0.41	34.53 ± 0.58[b]	36.80 ± 0.31[¶]
DPG	3.85 ± 0.16	3.24 ± 0.08[a]	5.00 ± 0.22[c,ψ]	3.09 ± 0.08	3.13 ± 0.15	5.80 ± 0.14[c,ψ]

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

Lyso: Lysophospholipid; SPM: sphinghomyelin; PC: phosphatidylcholine; PI: phosphatidylinositol; PS: phosphatidylserine; PE: phosphatidylethanolamine; DPG: diphosphatidylglycerol.

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

Table 9. Effects of alloxan diabetes and insulin treatment on phospholipid content in rat brain mitochondria.

Phospholipid Class	Content, μg / mg protein					
	One week			One month		
	Control	Diabetic	Diabetic + Insulin	Control	Diabetic	Diabetic + Insulin
Lyso	16.34 ± 0.94	5.22 ± 0.36[c]	8.97 ± 0.47[c,¶]	16.17 ± 0.85	15.03 ± 1.18	14.10 ± 0.49[c]
SPM	35.61 ± 1.04	18.84 ± 0.76[c]	37.44 ± 1.22[a,¶]	35.82 ± 1.11	37.18 ± 1.91[c]	34.96 ± 1.25[¶]
PC	187.80 ± 2.31	142.91 ± 3.87	179.79 ± 4.00	188.08 ± 2.45	188.57 ± 5.29[c]	168.55 ± 3.57[c,¶]
PI	17.41 ± 0.64	5.00 ± 0.30[c]	15.96 ± 1.17[¶]	17.54 ± 0.67	14.96 ± 0.95	9.23 ± 0.30[c,¶]
PS	21.56 ± 0.78	5.02 ± 0.26[c]	15.44 ± 1.02[¶]	21.62 ± 0.83	21.92 ± 1.91[c]	5.07 ± 0.17[c,¶]
PE	181.41 ± 3.41	131.95 ± 4.11[c]	163.35 ± 4.96[¶]	182.10 ± 3.64	159.33 ± 4.92[b]	148.58 ± 3.53[c,§]
DPG	18.45 ± 0.82	10.33 ± 0.37[b]	22.25 ± 1.21[¶]	18.74 ± 0.83	14.13 ± 0.82[c]	23.41 ± 0.70[c,ψ]

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

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

Table 10. Effects of alloxan-diabetes and insulin treatment on fluidity parameters of brain mitochondria.

Groups	Treatment	Fluidity Parameters			
		Fluorescence Polarization, p	Fluorescence anisotropy, r	Limited hindered anisotropy, rα	Order parameter, S
One week	Control	0.276 ± 0.003	0.203 ± 0.005	0.169 ± 0.007	0.652 ± 0.028
	Diabetic	0.245 ± 0.002[c]	0.178 ± 0.002[c]	0.138 ± 0.002	0.589 ± 0.004[c]
	Diabetic + Insulin	0.146 ± 0.001[c,¶]	0.102 ± 0.001[c,¶]	0.036 ± 0.001	0.299 ± 0.006
One month	Control	0.270 ± 0.004	0.197 ± 0.004	0.164 ± 0.006	0.649 ± 0.019
	Diabetic	0.286 ± 0.003[b]	0.211 ± 0.002[b]	0.181 ± 0.003[a]	0.673 ± 0.016
	Diabetic + Insulin	0.268 ± 0.002[¶]	0.196 ± 0.001[¶]	0.162 ± 0.002[¶]	0.636 ± 0.004[§]

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

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

phosphorylation in the brain mitochondria decreased significantly (9). Taken together, these observations corroborate the known fact that effects of diabetes on CNS are seen only at the later stage (5).

The results of the present studies point out that the early as well as late diabetic state created imbalance in the relative proportion of TPL and CHL. Insulin treatment was effective in restoring the relative proportion of the two lipid classes (Tables 2 and 3).

In the mitochondria, diabetic state had only marginal influence on the phospholipid composition and insulin treatment had an acidic phospholipids lowering effect. Also, insulin treatment significantly increased the content of DPG in both the diabetic groups. Previously it has been shown that the mitochondrial synthesis of DPG in the liver is regulated by thyroid hormones (9). Results of our present study would imply that at least in the brain mitochondria insulin may have regulatory role in DPG biosynthesis.

Changes in the gross parameters such as TPL and CHL content in whole brain, synaptic membranes and mitochondria as affected by diabetic condition have been reported (12-15). It also been demonstrated that the diabetic state affects the metabolism of glycolipids except for gangliosides and that the fatty acid composition of the phospholipid classes changes significantly (16).

The significant changes in the temperature kinetics properties of FoF_1 ATPase and lipid/phospholipid make-up of the brain mitochondrial membrane prompt us to carry out the correlation between kinetic parameter and phospholipid classes. The regression analysis data revealed that E_H showed negative correlation with PS and CHL ($r = -0.606$

and – 0.623 respectively) and positive correlation with ratio of TPL/CHL and TPL/PS (r = + 0.666 and 0.804 respectively). E_L showed negative correlation with DPG and ratio of DPG/basic phospholipids (BPL) (r = – 0.634 and – 0.648 respectively) while showing positive correlation with PC (r = – 0.616). Based on these results, it may suggested that that besides changes in the phospholipid composition the charge distribution across the membrane and especially in the lipid microdomains in which FoF_1 ATPase is embedded and changes in membrane fluidity may be a significant modulatory factor.

Summary

At late stage FoF_1 activity decreased by about 15 % and insulin treatment resulted in hyper-stimulation.

The brain mitochondrial enzyme in control animal showed sigmoidal substrate saturation curve i.e. allosteric pattern. In one month diabetic and insulin treated diabetic groups the typical Michaelis-Menten pattern was observed and corresponding Eadie-Hofstee plots displayed two component system.

In one month diabetic group treated with insulin Km values of both the components were low while Vmax values increased compared with the corresponding diabetic groups.

Hill plot analysis indicated that the Hill coefficients n_1 and n_2 decreased in late diabetic state and insulin treatment was ineffective in normalization. Up to 0.35 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.

From the temperature kinetics analysis it was noted that in control animals the enzyme activity displayed two phase transition temperatures (T_{t1} and T_{t2}) and three values for energy of activation (E_H, E_I and E_L), whereas in both diabetic and insulin treated diabetic groups only one T_t and two values for energies of activation (E_H and E_L) were noted.

In one week diabetic group the TPL and the CHL content decreased significantly whereas no change was observed in one month diabetic group. Insulin treatment in one week diabetic animals had only marginal effects on the TPL and no corrective effects on the CHL. The TPL and the CHL content decreased in one month diabetic animals after insulin treatment.

The phospholipid composition remained practically unchanged except in the one week diabetic animals where Lyso and DPG decreased while in one month diabetic group PE decreased. Insulin treatment in one week diabetic animals decreased Lyso and PS whereas DPG and SPM increased. In one month diabetic animals insulin treatment resulted in significant reduction in PI and PS composition and almost 2 fold increase in the DPG.

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