CHAPTER VI

EFFECTS OF CADMIUM TOXICITY ON THE OXIDATIVE ENERGY METABOLISM IN RAT BRAIN MITOCHONDRIA

INTRODUCTION

As outlined in the introductory section (Chapter I) Cd is known to be a neurotoxic trace metal [1]. There are some reports on the distribution of Cd in brain as compared to liver or kidneys as well as on the effect of Cd or Zn pre-treatment on this distribution. Presence of Cd in myelin and the effect exerted by Cd on neurotransmitter synthesis and metabolism have been studied [2,3]. The effect of Cd on brain has been presented as a cause-effect relationship between known Cd exposure and the observed subsequent neurological disorders. However , no concrete data regarding actual structural / functional alterations caused by Cd are available and the effect of Cd on mitochondria and oxidative metebolism in the brain remains an unexplored area.

Brain is a maximally protected organ as far as the exposure to Cd is concerned. As compared to the liver and the kidneys , only about 0.5% Cd accumulates in the brain [4,5]. The newborn immature rat brain is seen to have more Cd than the adult which is attributed to the incompletely developed blood-brain-barrier in the newborn as compared to the adult.

Studies outlined in the preceeding chapter (Chapter V) have shown that the Cd effect on oxidative energy metabolism in the liver mitochondria in young and adult rats was different in terms of the intensity as well as the time after which the adverse effect were evident.

In view of this it was of interest to extend the observations to examine the effect of Cd on the maximally protected organ i.e. the brain. Studies were hence carried out using young and adult rats; this was important since as can be seen from the results in Chapter III and as reported by Rajan and Katyare [6] brain oxidative phosphorylation and other enzymes of the energy metabolism in the brain exhibit a definite developmental pattern which is different from that seen for the liver.

Since the blood-brain-barrier restircts entry of Cd into the brain a time point in the early postnatal stage was selected, when the barrier is not completely closed, to inject Cd. This would ensure entry of Cd into brain which would then be able to exert effect on the mitochondrial energy metabolism.

Results of these experiments are descrined in this Chapter.

MATERIALS AND METHODS

Chemicals :

The chemicals and reagents used were the same as described in Chapter II and III.

Animals and Cd injections :

In these experiments rat pups of 7 days age and adults (8 to 10 week) were used. Cd dose and mode of injection was similar to that described in Chapter V. However, here the time after which the rats were killed differed.

The 1 week old rats were killed either 2 weeks (Cd-treated - 2 week group) or 2 months (Cd-treated - 2 month group) after Cd injection.

In case of adults in addition to the groups corresponding to those stated above i.e the Cd-treated-2 week and 2 month groups, another group i.e Cd-treated 48 h group also was kept, where the rats were killed 2 days after the Cd injection. With each experimental group, corresponding age matched controls were also maintained.

As in the case of liver (Chapter V) the control values for 2 month Cd-treated- 1 week group and adult controls corresponding to 48 h, 2 week and 2 months experimental groups were in close agreement. These were pooled and are given as the pooled controls in all the tables.

Details of other methods are the same as in Chapter V.

Results

Tables 1-3 present data on the oxidative phosphorylation in the rat brain mitochondria using, glutamate, succinate and ascorbate + TMPD as the substrates respectively.

The young rats killed 2 weeks after Cd treatment have a 20 % higher state 3 respiration rate with glutamate, while the other parameters do not show any significant alterations. When succinate was used as a substrate, ADP/O ratio and subsequently ADP-phosphorylation rates decreased by 40%. With asocrbate + TMPD, ADP/O ratio and state 3 rates decreased by 25% and 13% respectively and ADP-phosphorylation rates were low (-40%) compared to the control. In the young rats killed 2 months after Cd treatment. with glutamate the state 3 respiration rate and ADP- phosphorylation rate decreased (55 to 65% decrease). With succinate also a more-or-less similar effect is seen. When ascorbate + TMPD was used, the Cd-treated group in addition to the decrease in the above parameters, also showed a decrease in the ADP/O ratio resulting in a substantial inhibition of the ADP-phosphorylation rate.

In the adult group killed 48 h after Cd treatment, the oxidative metabolism decreased with glutamate. With succinate the state 3 rate was stimulated (+15%). For ascorbate + TMPD a pattern similar to that with glutamate was seen, in that the ADP/O ratio and the ADP-phosphorylation decreased, however, the extent of decrease was greater (-50% and -40% respectively) In the adult group killed 2 weeks after Cd

Table 1

Effect of cadmium treatment on the oxidative phosphorylation in rat brain mitochondria with glutamate as the substrate.

Age	Treatment/ Duration	ADP/O ratio	Respiration rate State3	Respiration rate State 4	ADP-phosphorylation rate
1 Week	Control (12)	2.27 $\pm$ 0.16	6.4 $\pm$ 0.19	4.1 $\pm$ 0.38	30.0 $\pm$ 2.07
	Cd-treated - 2 Week (12)	2.26 $\pm$ 0.11	7.6 $\pm$ 0.25[a]	4.0 $\pm$ 0.36	32.3 $\pm$ 2.56
	Pooled Control (40)	3.01 $\pm$ 0.08	30.4 $\pm$ 0.96	8.4 $\pm$ 0.12	164.9 $\pm$ 0.83
	Cd-treated -2 Month (12)	3.20 $\pm$ 0.06	16.2 $\pm$ 1.75[a]	4.9 $\pm$ 0.38[a]	105.3 $\pm$ 6.01[a]
Adult	Pooled Control (40)	3.01 $\pm$ 0.08	30.4 $\pm$ 0.96	8.4 $\pm$ 0.12	164.9 $\pm$ 0.83
	Cd-treated -48 h (12)	2.97 $\pm$ 0.20	20.5 $\pm$ 1.32[a]	5.4 $\pm$ 0.42[a]	140.1 $\pm$ 3.18[a]
	Cd-treated -2 Week (12)	2.93 $\pm$ 0.15	24.8 $\pm$ 1.20[a]	2.6 $\pm$ 0.25[a]	158.4 $\pm$ 2.68
	Cd-treated -2 Month (12)	2.78 $\pm$0.08	13.3 $\pm$ 0.47[a]	2.1 $\pm$ 0.13[a]	105.0 $\pm$ 4.00[a]

continued on next page .

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

Control- the age matched control (as described in the text)

Pooled control- as described in the text

Cd-treated

48 h group - experimental group killed 48 hours after a single i. p. Cd injection.

2 week group - experimental group killed two weeks after a single i. p. Cd injection.

2 month group - experimental group killed two months after a single i. p. Cd injection

ADP / O ratio - n moles of ADP phosphorylated / n atoms of O_2 consumed.

Respiration rate - n moles of O_2 consumed / min / mg mitochondrial protein.

ADP - phosphorylation rates - nmoles of ATP formed / min / mg mitochondrial protein.

[a] $P < 0.001$ compared to the corresponding controls.

Table 2

Effect of cadmium treatment on the oxidative phosphorylation in rat brain mitochondria with succinate as the substrate.

Age	Treatment/ Duration	ADP/O ratio	Respiration rate State3	Respiration rate State 4	ADP-phosphorylation rate
1 Week	Control (12)	0.81 ± 0.04	40.8 ± 1.43	31.2 ± 1.55	66.4 ± 1.88
	Cd-treated -2 Week (12)	0.44 ± 0.01[b]	43.1 ± 1.49	28.7 ± 1.89	39.5 ± 0.92[b]
	Pooled Control (40)	1.06 ± 0.08	64.1 ± 0.98	34.6 ± 1.00	201.1 ± 5.15
	Cd-treated -2 Month (12)	1.62 ± 0.20	37.0 ± 3.55[b]	24.8 ± 1.80[b]	119.0 ± 9.15[b]
Adult	Pooled Control (40)	1.66 ± 0.08	64.1 ± 0.98	34.6 ± 1.00	201.1 ± 5.15
	Cd-treated -48 h (12)	1.54 ± 0.11	73.9 ± 2.61[b]	45.9 ± 2.06[b]	220.5 ± 10.30
	Cd-treated -2 Week (12)	0.56 ± 0.05[b]	95.8 ± 5.18[b]	29.6 ± 2.06[a]	110.5 ± 7.19[b]
	Cd-treated -2 Month (12)	1.49 ± 0.06	43.4 ± 3.02[b]	11.5 ± 1.24[b]	135.8 ± 6.90[b]

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

Other details are as given in Table 1

[a]$P < 0.05$; [b]$P < 0.001$ compared to the corresponding controls.

Table 3

Effect of cadmium treatment on the oxidative phosphorylation in rat brain mitochondria with ascorbate + TMPD as the electron donor system.

Age	Treatment/ Duration	ADP/O ratio	Respiration rate state3	Respiration rate state 4	ADP-phosphorylation rate
1 Week	Control (12)	0.33 ± 0.02	59.6 ± 2.73	15.7 ± 1.30	39.8 ± 1.33
	Cd-treated -2 Week (12)	0.25 ± 0.01^{b}	52.3 ± 2.16^{a}	13.6 ± 1.05	25.4 ± 0.61^{c}
	Pooled Control (40)	0.48 ± 0.01	179.3 ± 5.11	71.4 ± 1.12	158.7 ± 3.78
	Cd-treated -2 Month (12)	0.28 ± 0.01	89.5 ± 6.35^{c}	52.7 ± 2.22^{c}	52.9 ± 3.21^{c}
Adult	Pooled Control (40)	0.48 ± 0.01	179.3 ± 5.11	71.4 ± 1.12	158.7 ± 3.78
	Cd-treated -48 h (12)	0.26 ± 0.01^{c}	186.1 ± 12.5	42.4 ± 2.83^{c}	94.1 ± 10.3^{c}
	Cd-treated -2 Week (12)	0.34 ± 0.01^{c}	131.0 ± 4.04^{c}	44.1 ± 5.47^{c}	81.4 ± 5.36^{c}
	Cd-treated -2 Month (12)	0.33 ± 0.01^{c}	107.4 ± 7.01^{c}	44.5 ± 3.00	3.2 ± 0.2^{c}

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

Other details are as given in Table 1

$^{a}P < 0.05$; $^{b}P < 0.002$; $^{c}P < 0.001$ compared to the corresponding controls.

Table 4

Effect of cadmium treatment on rat brain mitochondrial dehydrogenases.

Age	Treatment/ Duration	Glutamate dehydrogenase	Succinate-DCIP reductase
1 Week	Control (12)	3.8 ± 0.15	18.3 ± 1.54
	Cd-treated -48 h (10)	2.6 ± 0.05[a]	27.1 ± 2.35[a]
	Pooled Control (12)	25.3 ± 0.04	20.6 ± 0.06
	Cd-treated -2 Month(10)	37.1 ± 0.33[a]	42.7 ± 1.47[a]
Adult	Pooled Control (12)	25.3 ± 0.04	20.6 ± 0.06
	Cd-treated -48 h (10)	26.4 ± 0.61	44.2 ± 0.79[a]
	Cd-treated -2 Week (16)	12.8 ± 0.85[a]	33.4 ± 0.92[a]
	Cd-treated -2 Month(10)	25.1 ± 0.89	22.7 ± 0.53

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

Other details are as given in Table 1

Enzyme activity - n moles/min/mg mitochondrial protein

[a] $P < 0.001$ compared to the corresponding controls.

Table 5

Effect of cadmium treatment on rat brain mitochondrial ATPase activity.

Age	Treatment/ Duration	ATPase activity			
		Basal	Mg^{2+}	DNP	Mg^{2+} + DNP
1 week	Control (16)	3.5 ± 0.12	6.8 ± 0.26	2.6 ± 0.12	14.3 ± 0.57
	Cd-treated -2 Week (16)	2.8 ± 0.23[a]	13.5 ± 0.47[c]	3.2 ± 0.12[b]	12.7 ± 0.25[a]
	Pooled Control (40)	1.9 ± 0.02	9.3 ± 0.05	2.8 ± 0.03	17.3 ± 0.08
	Cd-treated -2 Month(16)	2.6 ± 0.13[c]	12.2 ± 0.21[c]	2.4 ± 0.29	12.4 ± 0.28[c]
Adult	Pooled Control (40)	1.9 ± 0.02	9.3 ± 0.05	2.8 ± 0.03	17.3 ± 0.08
	Cd-treated -48 h (16)	1.4 ± 0.12[c]	6.5 ± 0.19[c]	4.7 ± 0.28[c]	16.6 ± 0.34
	Cd-treated -2 Week (16)	1.6 ± 0.07[c]	6.9 ± 0.20[c]	1.2 ± 0.07[c]	11.1 ± 0.14[c]
	Cd-treated -2 Month(16)	1.4 ± 0.07[c]	7.0 ± 0.26[c]	1.6 ± 0.10[c]	12.3 ± 0.36[c]

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

Other details are as given in Table 1

ATPase activity - µmoles Pi liberated /h./mg mitochondrial protein

[a]$P < 0.02$; [b]$P < 0.002$; [c]$P < 0.001$ compared to the corresponding controls.

Table 6

Effect of cadmium treatment on the soluble and total -SH content in rat brain mitochondria.

Age	Treatment/ Duration	- SH Content	
		Soluble	Total
1 Week	Control (16)	-----	88.1 ± 1.71
	Cd-treated -2 Week (16)	-----	77.8 ± 1.92[a]
	Pooled Control (40)	-----	80.9 ± 0.53
	Cd-treated -2 Month(16)	-----	16.6 ± 0.01[a]
Adult	Pooled Control (40)	-----	80.9 ± 0.53
	Cd-treated - 48 h (16)	------	51.5 ± 1.31[a]
	Cd-treated -2 Week (16)	------	60.9 ± 1.84[a]
	Cd-treated -2 Month(16)	------	51.8 ± 2.10[a]

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

Other details are as given in Table 1

-SH content - n moles/mg mitochondrial protein.

[a]P < 0.001 compared to the corresponding controls.

treatment, oxidative phosphorylation with glutamate did not improve. With succinate the ADP/O ratio was very drastically impaired to the extent of 70%, while state 3 rate was actually stimulated by about 50%. Due to the impaired ADP/O ratio the ADP-phosphorylation rate was about half the control value. With ascorbate + TMPD, state 3 respiration decreased with parallel decrease in the ADP-phosphorylation rate.

In the last experimental group i.e. the adult rats killed 2 months after Cd treatment, there was further reduction in state 3 respiration rate and ADP-phosphorylation rate with glutamate. Similar results were seen with succinate and ascorbate + TMPD.

The changes occuring in the GDH and SDR activities are shown in table 4. In the young rats killed 2 weeks after Cd treatment, the GDH activity was impaired and had a lower value of about 70% of the control. SDR activity, however, gets stimulated and was about 50% higher. In the young rats killed two months after Cd treatment both GDH (+47%) and SDR (+2fold) had higher activities.

In the adult rats, killed 48 h after Cd treatment GDH was unaffected while SDR was stimulated 2 fold. In the rats killed 2 weeks after Cd treatment GDH activity was impaired (-50%)while the SDR activity was still 62% higher than the control. In the adult rats killed 2 months after Cd treatment

the GDH activity recovers and becomes similar to the control while SDR activity also returns to the control levels.

The basal ATPase activity in the young rats killed 2 weeks after Cd treatment (Table 5) is reduced by 20% while the Mg^{2+} stimulated ATPase was about 2 fold higher in the Cd treated groups as compared to the controls. The DNP ATPase also was stimulated though only by 23%. However the combined Mg^{2+} + DNP ATPase activity was stimulated by about 35% while the DNP ATPase activity was reduced (-15% and -10% respectively).

In the adults the 48 h Cd treated group was seen to have a reduced activity as far as the basal and Mg^{2+} ATPase are concerned (-26% and - 30% respectively) while the Mg^{2+} ATPase activity was 70% stimulted. In the 2-week Cd-treated group the presence of Cd was seen to cause inhibition irrespective of the stimulatory condition : 16% in basal, 25% in Mg^{2+} and 60% in DNP and Mg^{2+} + DNP ATPase. In the adult 2 month group also Cd seemed to cause inhibiton of ATPase activity, about 30% for all except DNP ATPAse where the effect was the highest, about -40%.

The effect of Cd treatment on the -SH group content is shown in table 6. As can be seen, the soluble - SH groups were not detectable in all the groups, experimental as well as the control. As far as the total -SH content was concerned, the young rats killed 2 weeks after Cd treatment showed a 12%

decrease in content while the 2 month group had a 80% reduction in the total-SH content in the Cd-treated group. The adult group 48-h Cd-treated and 2-month Cd-treated each had about 36% reduction in -SH content while the 2 weeks Cd-treated group has about 25% reduction.

DISCUSSION

The brain mitochondria from young rats exposed to Cd were more resistant to the Cd-insult than the mitochondria from adult. This is evident from the fact that in young rats the killed two weeks after Cd-treatment, the brain mitochondrial respiratory activity was stimulated with almost all the three substrates though to a varying degree. However in the adults the similar experimental group showed inhibiton of respiratory activity. Thus as was seen with the liver, brain also has a tendency to be more resistant to Cd-induced damage in the young stage as against the adults. This could be mostly due the same reason as with liver i.e. brain, as seen in Chapter III (also see Rajan and Katyare [6]), shows a constantly increasing trend of the mitochondrial oxidative phosphorlation and ETC related enzymes with development and hence by some mechanism which as yet remains unclear, the mitochondria are able to assess and rectify the damage which may have been caused by Cd and in doing so overshoot the control level.

Wong *et.al*. [7] have shown that immature rat brain contains more Cd as compared to the adults mostly due to improperly developed blood-brain-barrier in the young. However, another important fact is that the toxicity of Cd in a tissue does not always correlate with the concentration of Cd in that tissue. An example is the testes, where even though the concentration of Cd in the testes was more in the immature as compared to the adult rats, due, as in case of brain, to the incompletely developed blood-testis-barrier, the testes of the immature rats were resistant to the damaging

effect of Cd while those of the adults were not. This suggests that the Cd level is not the only factor in determining Cd toxicity, but the stage of the organ development could also be an important factor [7].

Similar to liver, brain also shows inhibition of the membrane bound enzymes in the 48 h period in adult rats where the maximum mitochondiral membrane damage is seen [8] in liver mitochondria from adult rats.

The two month group in both the young and the adults also has lower respiratory activity as compared to the control. Thus the 2 week period seems to be a period where the brain mitochondria in the Cd-treated groups are making on attempt to recover from the damage caused by the Cd insult. However, after a longer period of time i.e. 2 months the activity is once again inhibited and the damage becomes more pronounced. Thus as with liver, oxidative phosphorylation in brain also seems to succumb to Cd damage, after a longer period of time has elapsed.

Depending on the time elapsed after Cd-treatment the experimental groups showed differences in the effects on the respiratory activity. Respiration with glutamate was impaired within 48 h, while with succinate the rate was slightly stimulated. In the 2 week group the activity with glutamate and succinate was stimulated while that with ascorbate + TMPD was impaired. Only after 2 month period of Cd treatment the

respiratory activity with all the three substrates was inhibited. The results thus pointed out that after passage of a longer period of time the respiratory activity in general is adversely affected and impaired.

Of the dehydrogenases GDH is seen to be most affected in the 2 week period in both the young and the adults while the 2 month group either shows stimulation as in the young or similar values to the control as in the adults and the activity in the 48 h also remains constant. SDR on the whole shows a stimulated activity in presence of Cd in almost all the groups, except the adult group killed 2 months after Cd treatment where the values are similar to the controls. Thus the dehydrogenases in brain do not seem to be too drastically inhibited. However, since the activity especially of SDR was reduced from a stimulated high value to a value similar to the control there is a possibility that further period would cause reduction in activity.

In case of ATPase the basal and Mg^{2+} + DNP ATPase is most susceptible to Cd-insult since in all the experimental groups it is well below the control values. While Mg^{2+} and DNP when present separately are able to stimulate the activity in the earlier periods and while in the 2 month period, especially for the adults, Cd seems to cause inhibition of the enzyme activity.

Thus on the whole the adult rat brain mitochondria seem to be more susceptible to Cd-insult as compared to the young rats. Since Klaassen and Wong [9] have shown that in the newborn the content of MT is 20 times more than in the adults this could be compensating for the larger amount of Cd that could be able to get through into the brain since the blood-brain-barrier, at the time of injection, was still not closed. In fact, in the 2-week-group of the young rat the maximum content of -SH (total) was seen amongst all the experimental groups. On the otherhand, the young and adult rats killed 2 months after the Cd injection had the least total -SH content and this could be a reason why the long term exposure groups showed greater damage to the activity.

Thus in conclusion the damage to the rat brain mitochondria seen in the 2 month i.e. the long-term treatment groups could give a clue to the known pathophysiological and neuropathological conditions, which make Cd a neurotoxic trace metal. Since these effects are seen after a long period of time, mostly years after continous exposure to Cd, this delayed derangement of mitochondrial oxidative metabolism and related enzymes may give an insight into its mode of action.

SUMMARY

The effect of Cd treatment on brain mitochondria in terms of the activities of oxidative phosphorylation and related enzymes was studied in young (1 week) and adult (8 to 10 week) rats.

The Cd effects were checked 2 weeks and 2 months, in case both young and adults, and additionally at 48 h, in the adults, after the single Cd i.p. injection.

In both the age groups the oxidative phosphorylation was relatively unaffected in the 2-week group while in comparison, the 2 month group had suffered much more damage to the activity. In case of the 48 h gr in adults this too had impaired activity.

GDH activity was unaffected in the 2 months and 48 h groups, while only the 2 week group, in both young and adults, showed inhibition. SDR activity was in fact stimulated in presence of Cd in all groups except the adults 2 month group.

ATPase activity on the whole showed inhibition although the levels of inhibition varied depending on the stimulatory condition.

The soluble -SH groups were undetectable in all the groups, while the total -SH content in the 2 week groups was maximum and the 2 month group the minimum.

Thus the adverse affect of Cd seen after the 2 month period could give an insight regarding the late onset and manifestation of the neuropathological effects of Cd.

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