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Pharmacology, Biochemistry and Behavior 70 (2001) 325–331

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Effect of chronic treatment of melatonin on learning, memory and oxidative deficiencies induced by intracerebroventricular streptozotocin in rats

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Received 21 November 2000; received in revised form 5 June 2001; accepted 29 June 2001

Abstract

Intracerebroventricular (ICV) streptozotocin (STZ) has been shown to cause cognitive impairment, which is associated with free radical generation in the brain of rats. Melatonin is a potent free radical scavenger and antioxidant. In the present study, the effect of melatonin was investigated against ICV STZ induced cognitive impairment and oxidative stress in rats. Adult male Wistar rats were injected with ICV STZ (3 mg/kg) bilaterally. The rats were treated with STZ twice, on days 1 and 3. The learning and memory behavior was assessed using passive avoidance paradigms, elevated plus maze and the closed field activity while the parameters of oxidative stress assessed were malondialdehyde (MDA) and glutathione. The rats were treated chronically with melatonin for 21 days starting from day 1 of STZ injection. The learning and memory behavior was evaluated on days 17, 18 and 19 and the rats were sacrificed on day 21 for estimation of MDA and glutathione. The rats treated with melatonin showed significantly less cognitive impairment. There was also insignificant increase in brain MDA and decrease in glutathione levels in melatonin-treated ICV STZ rats as compared to the vehicle-treated ICV STZ animals. The study demonstrates the effectiveness of melatonin in preventing the cognitive deficits as well as the oxidative stress caused by ICV STZ in rats and suggests it's potential in age and age-related neurodegenerative disorders where oxidative stress and cognitive impairment are involved.

Keywords: Streptozotocin; Melatonin; Rat; Oxidative stress

1. Introduction

As the world's population is growing increasingly older, there is an increase in both life expectancy and in age related neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. It is well known that both aging and age associated neurodegenerative disorders are associated with varying degrees of behavioral impairment that cause significant morbidity (Cantuti-Castelvetri et al., 2000).

Among the prime candidates responsible for producing the neuronal changes mediating these behavioral deficits, appear to be free radicals and the oxidative stress they generate. Therefore, free radical scavengers and antioxidants have been proposed as agents that may delay or inhibit the progression of such neurodegenerative disorders (Joseph et al., 1999).

Melatonin, the primary secretory product of the pineal gland, is known to possess free radical scavenging and antioxidant properties (Reiter et al., 1997). Evidence suggests that the chronic prophylactic administration of melatonin as a gerontoprotector is based on its antioxidant properties (Reiter et al., 1996). Numerous other studies also indicate that melatonin as a free radical scavenger displays pronounced neuroprotective effects against excitatory amino acids and toxic effects of beta amyloid peptide—one of the specific hallmarks of Alzheimer's disease (Papolla et al., 1997, 2000).

Intracerebroventricular (ICV) injection of streptozotocin (STZ), in a sub diabetogenic dose in rat has been found to cause prolonged impairment of brain glucose and energy metabolism. This is accompanied by impairment in learning and memory in addition to decreased choline acetyltransferase levels in the hippocampus (Blokland and Jolles, 1993; Lannert and Hoyer, 1998). We have recently shown the presence of oxidative stress

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0091-3057/01/$ – see front matter
PII: S0091-3057(01)00611-6

i.e. an increase in lipid peroxidation and decrease in the antioxidant glutathione in rat brains following ICV STZ injection along with cognitive impairment (Sharma and Gupta, 2001).

Therefore, the present study was undertaken to evaluate the effect of melatonin for its effect on learning and memory and on markers of oxidative stress after ICV STZ in rats.

2. Methods

2.1. Animals

Adult male Wistar rats weighing 320–350 g were used. The animals were obtained from the central animal facility of All India Institute of Medical Sciences, New Delhi and stock bred in the departmental animal house. The rats were group housed in polyacrylic cages ($38 \times 23 \times 10$ cm) with not more than four animals per cage and maintained under standard laboratory conditions with natural dark and light cycle. They were allowed free access to standard dry rat diet and tap water ad libitum. All procedures described were reviewed and approved by the Institutional Committee for Ethical Use of Animals.

2.2. Experimental protocol and drug

Melatonin (Courtesy, Dabur, India) was prepared in propylene glycol. It was administered chronically for 21 days between 10 and 11.00 a.m. every day at doses of 10 and of 20 mg/kg ip starting from day of 1st STZ ICV injection to day 21; when the rats were sacrificed for MDA and glutathione estimation. On the day of the ICV injections (days 1 and 3), melatonin was injected just before the ICV injection. The dose of melatonin was selected on the basis of earlier reports in which significant antioxidant property was demonstrated in doses ranging from 0.1 to 10 mg/kg (Raghavendra and Kulkarni, 2001) and 10 and 20 mg/kg (Ustundag et al., 2000).

2.3. Intracerebroventricular injection of streptozotocin

Rats were anesthetized with ketamine hydrochloride (Themis, India) at a dose of 70 mg/kg ip. The head was positioned in a stereotactic frame and a midline saggital incision was made in the scalp. Burr holes were drilled in the skull on both the sides over the lateral ventricles using the following coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to saggital suture, 3.6 mm beneath the surface of brain. The STZ group; vehicle- and melatonin-treated, were given a bilateral ICV injection of STZ (Sigma, St. Louis, USA) (3 mg/kg body weight). STZ was dissolved in artificial CSF and a solution of 25 mg/ml was made. This solution was made freshly and just before the injection. Each rat was given 20-μl injection on each site. The volume of the injection was kept constant, as the variation in the amount of drug in each rat was minimum as the rats were of a close weight range. The injection (3 mg/kg) was repeated on day 3. In the sham group, artificial CSF: 147 mM NaCl, 2.9 mM KCl, 1.6 mM $MgCl_2$, 1.7 mM $CaCl_2$ and 2.2 mM dextrose was injected (20 μl on each site) on the same days as in STZ group. Post Operatively, the rats were fed sweetened milk (1.5 ml) orally using an intra gastric tube once a day for 4 days following the surgery or till they started spontaneous feeding.

2.4. Effect on learning and memory (behavioral tests)

For the behavioral tests each group comprised of 10 rats and during behavioral testing only one animal was tested at a time.

2.5. One trial passive avoidance task

Memory retention deficit was evaluated by a step through passive avoidance apparatus according to the method previously described (Nakahara et al., 1998) on days 17 and 18 after 1st injection of STZ. Briefly, on the acquisition trial, each rat was placed in the lighted chamber. After 60 s of habituation period, the guillotine door separating the lighted and dark chamber was opened, and the initial latency (IL) to enter the dark chamber was recorded. Rats that had an initial latency time of more than 60 s were excluded from further experiments. Immediately after the rat entered the dark chamber, the guillotine door was closed and an electric foot shock (75 V, 0.2 mA, 50 Hz) was delivered to the floor grids for 3 s. Five seconds later, the rat was removed from the dark chamber and returned to its home cage. Twenty-four hours later, the retention latency (RL) time was measured in the same way as in the acquisition trial, but foot shock was not delivered, and the latency time was recorded to a maximum of 600 s.

2.6. Elevated plus maze

Acquisition and retention of memory processes was assessed using elevated plus maze on days 18 and 19 of STZ injection (Sharma and Kulkarni, 1992). The rat is placed on the open arm facing outwards and the transfer latency (TL) (the time in which the rat moves from the open arm to the closed arms) is noted. On the next day, the rat is placed similarly on the open arm and the TL is noted again.

3. Closed field activity

Spontaneous locomotor activity was assessed on day 19 after STZ administration. Each animal was observed

over a period of 300 s in square closed arena equipped with infrared light sensitive photocells using a digital photoactometer (Techno, India). The apparatus was housed in a darkened light and sound attenuated ventilated testing room.

3.1. Estimation of oxidative stress parameters

For the biochemical tests, each group comprised of 10 rats. Lipid peroxidation was estimated in the brain on the 21st day after STZ injection. Glutathione was also estimated on day 21. Simultaneous sham experiments were run. The rats were decapitated under ether anesthesia and the brains quickly removed, cleaned with chilled saline and stored at − 80 °C until biochemical analysis which were done within the next 7 days.

3.2. Measurement of lipid peroxidation

MDA, which is a measure of lipid peroxidation, was measured spectrophotometrically (Okhawa et al., 1979). Briefly, brain tissues were homogenized with 10 times (w/v) 0.1 M sodium phosphate buffer (pH 7.4). The reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of processed tissue sample. The mixture was then heated at 100 °C for 60 min. The mixture was cooled with tap water and 5 ml of *n*-butanol:pyridine (15:1% v/v), 1 ml of distilled water was added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was withdrawn and absorbance was measured at 532 nm using a spectrophotometer.

3.3. Measurement of glutathione

Glutathione was measured spectrophotometrically (Ellman, 1959). Briefly, brain tissues were homogenized with 10 times (w/v) 0.1 M sodium phosphate buffer (pH 7.4). This homogenate was then centrifuged with 5% trichloroacetic acid to centrifuge out the proteins. To 0.1 ml of this homogenate, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5′5-dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of double distilled water was added. The mixture was vortexed and the absorbance read at 412 nm within 15 min.

3.4. Statistical analysis

The results are expressed as mean + S.E.M. Statistical analysis of the passive avoidance, elevated plus maze and the locomotor activity values were performed by means of the Mann–Whitney *U* nonparametric tests. The statistical difference of the biochemical data was also evaluated by the same test. * $P < .05$ represents level of significance.

4. Results

4.1. Effect of melatonin on parameters of learning and memory

4.1.1. Passive avoidance task

The mean initial latency on day 17 did not differ significantly between the sham, vehicle-treated ICV STZ group and the melatonin 10 and 20 mg/kg ip treated ICV STZ group. The initial latency was 13.5 ± 1.67, 15.3 ± 2.6, 11.83 ± 3.15 and 17 ± 4.96 s.

On day 18, the mean retention latency in vehicle-treated ICV STZ group was significantly less 135 ± 40 ($P < .05$) as compared to that of sham rats (530 ± 23.9). The group that was treated with melatonin, both 10 and 20 mg/kg ip, showed significant reversal ($P < .05$) of transfer latency. The mean retention latency was 290.75 ± 51.45 and 288.5 ± 12.98 s, respectively, which was significantly higher than vehicle-treated STZ group indicating improved acquisition or retention of memory. There was insignificant difference between the melatonin 10 and 20 mg/kg dose treatment (Fig. 1).

4.1.2. Elevated plus maze

There was insignificant difference in the initial transfer latency (day 18) of the sham, vehicle-treated ICV STZ group and the melatonin-treated (10 and 20 mg/kg ip) ICV STZ group. The initial transfer latencies were 54.34 ± 3.14, 47.9 ± 2.78, 43.16 ± 4.92 and 56.6 ± 6.54 s, respectively. The retention transfer latencies on day 19 were 26 ± 4.2, 49 ± 4, 21.36 ± 6.86 and 27.3 ± 7.1 s, respectively. There was a significant difference ($P < .05$) between the initial and retention transfer latency in the sham group and melatonin (10 and 20 mg/kg ip) ICV STZ group respectively signifying acquisition and retention of memory, while the difference between the initial and retention transfer latencies in the vehicle-treated ICV STZ group was insignificant (Fig. 2).

4.1.3. Closed field activity

The spontaneous locomotor activity did not differ significantly between the sham, vehicle-treated ICV STZ group and melatonin-treated ICV STZ group on day 19. The mean values in the sham, vehicle-treated ICV STZ and melatonin (10 and 20 mg/kg ip)-treated ICV STZ group were 190 ± 15, 184 ± 19, 188.6 ± 15.83 and 189.83 ± 11.73 s.

4.2. Estimation of parameters of oxidative stress

4.2.1. Rat brain MDA level

The brain MDA levels were estimated on day 21. The vehicle-treated ICV STZ rats showed significant ($P < .05$) rise in levels of MDA as compared to the sham group. The MDA levels for the vehicle-treated ICV STZ and sham group were and 530 ± 30 and 166 ± 22.3 nmol/g wet tissue, respectively. The ICV STZ groups, which were treated with melatonin for 21 days, did not show any significant rise in

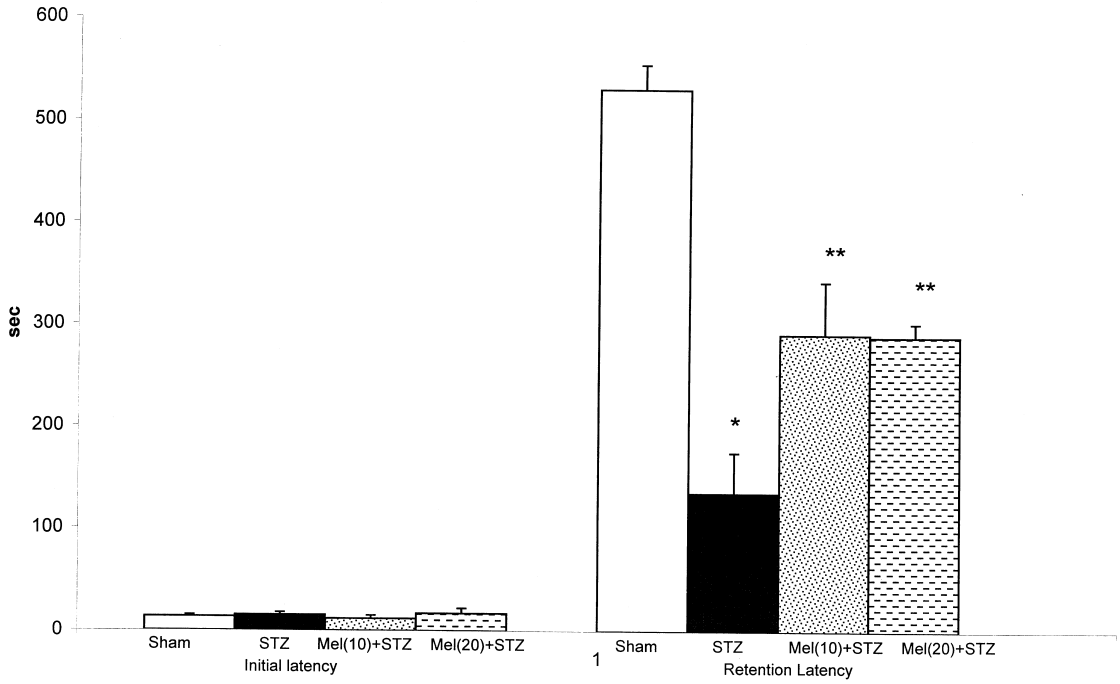

Fig. 1. Effect of chronic treatment with melatonin (10 and 20 mg/kg ip) on passive avoidance paradigm in rats. Values are expressed as mean ± S.E.M. * $P < .05$ retention latency of vehicle-treated ICV STZ rats vs. retention latency of sham. ** $P < .05$ vehicle-treated STZ retention latency vs. retention latency of melatonin (10 and 20 mg/kg) treated rats.

MDA level as compared to the sham group. The MDA values of the melatonin (10 and 20 mg/kg ip)-treated ICV STZ were 300 ± 55.04 and 218.33 ± 54.12 nmol/g wet tissue, respectively (Fig. 3).

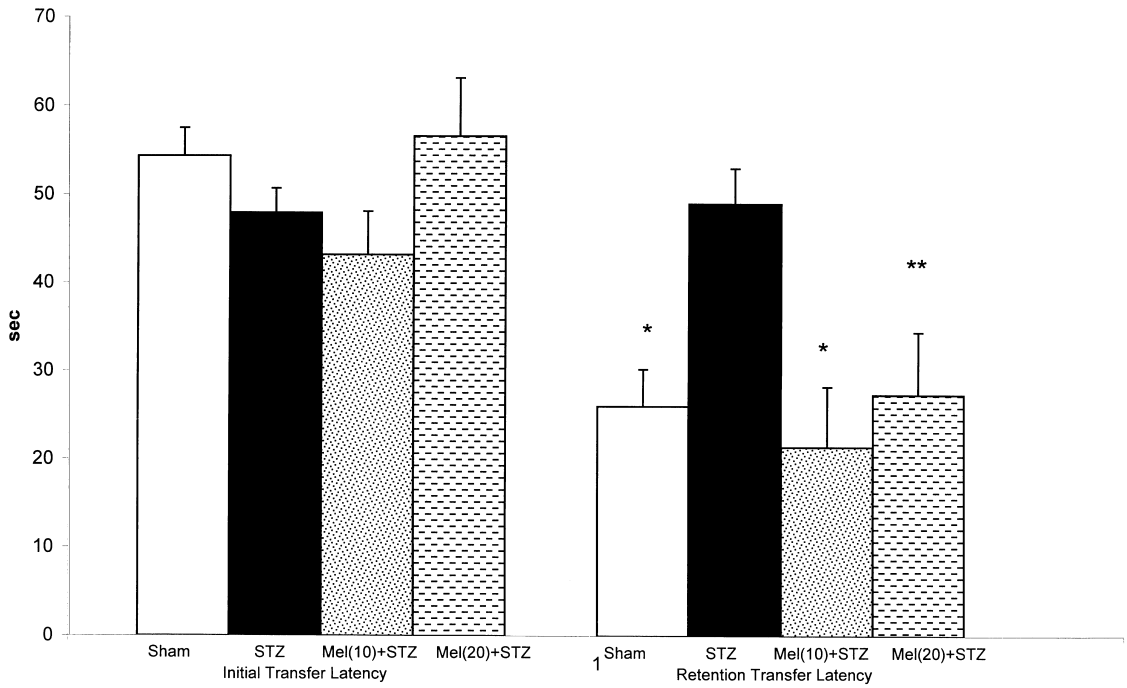

Fig. 2. Effect of chronic treatment of melatonin (10 and 20 mg/kg ip) on elevated plus-maze test in rats. Values are expressed as mean ± S.E.M. * $P < .05$ initial transfer latency of sham vs. sham retention transfer latency. ** $P > .05$ initial transfer latency of melatonin ICV STZ rats vs. melatonin ICV STZ retention transfer latency.

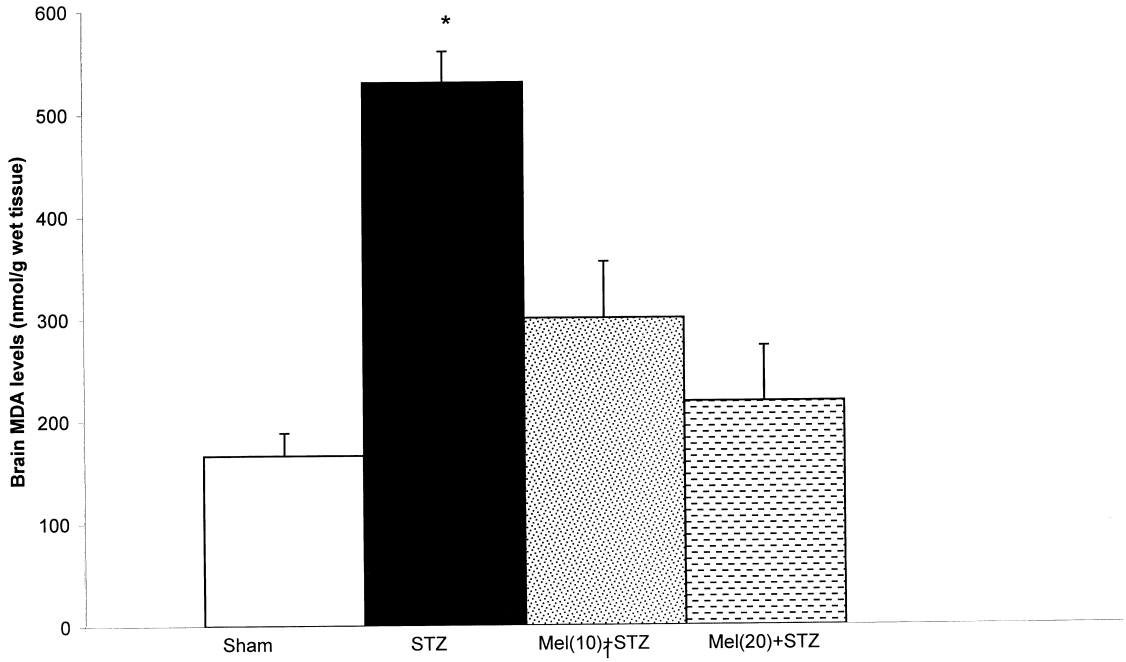

Fig. 3. Effect of chronic treatment with melatonin (10 and 20 mg/kg ip) on MDA levels in rat brains. Values are expressed as mean ± S.E.M. * $P < .05$ vehicle-treated ICV STZ vs. sham.

4.2.2. Rat brain glutathione levels

Glutathione was estimated on day 21. There was a significant ($P < .05$) fall in the levels of glutathione in the vehicle-treated ICV STZ group as compared to the sham group on day 21. The values of sham group and vehicle-treated STZ group on day 21 was 467 ± 18 μg/g tissue as compared to 313 ± 15 μg/g tissue, respectively. The glutathione value of the melatonin (10 and 20 mg/kg)-treated

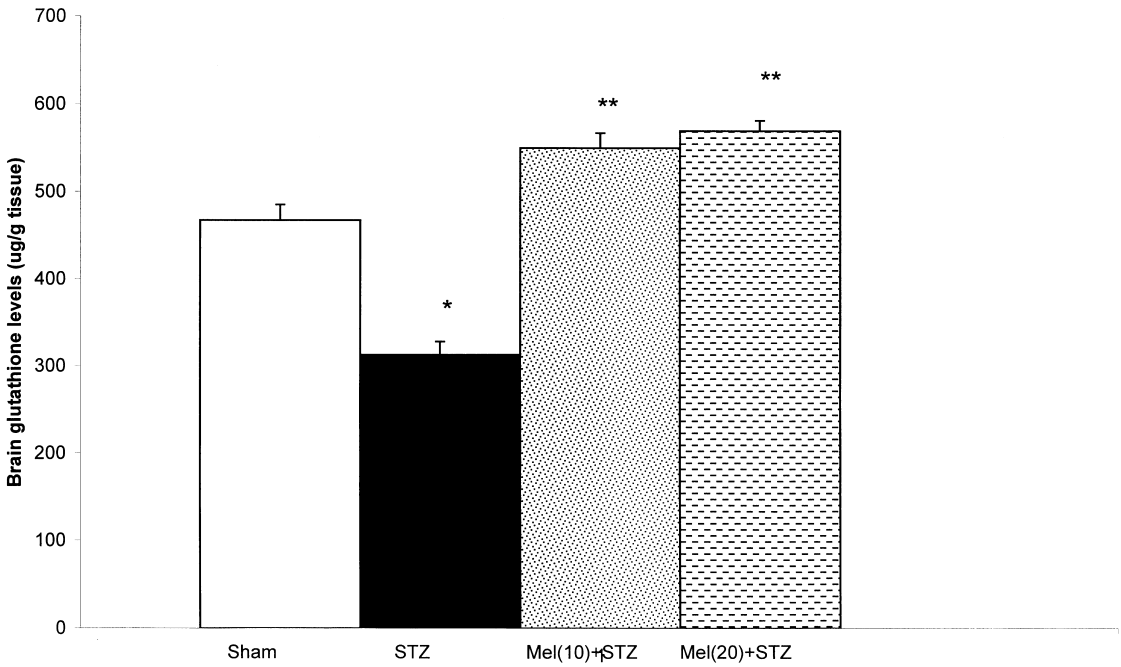

Fig. 4. Effect of chronic treatment with melatonin (10 and 20 mg/kg ip) on glutathione levels in rat brains. Values are expressed as mean ± S.E.M. * $P < .05$ vehicle-treated ICV STZ vs. sham. ** $P < .05$ melatonin ICV STZ rats vs. sham.

ICV STZ group was significantly higher ($P < .05$) that that of the vehicle-treated ICV STZ group and the sham group. The values being 549.6 ± 17 and 569 ± 12 μg/g tissue, respectively (Fig. 4).

5. Discussion

Melatonin has been shown to be highly effective in reducing oxidative damage in the central nervous system; this efficacy derives from its ability to directly scavenge a number of free radicals and to function as an indirect antioxidant (Reiter et al., 1999). Since melatonin crosses the blood brain barrier with ease and enters cells and subcellular compartments (Reiter et al., 1997), we found it worthwhile to investigate whether melatonin has a protective role against ICV STZ cognitive impairment as well as oxidative stress.

The ICV STZ model has been described as an appropriate animal model for sporadic Alzheimer type dementia (Lannert et al., 1998) and is characterized by a progressive deterioration of memory, cerebral glucose and energy metabolism and presence of oxidative stress (Lannert et al., 1998; Sharma and Gupta, 2001).

Since neuronal injury itself can induce free radical generation, it is difficult to establish whether this is a primary or secondary event. Even if free radical generation is secondary to other initiating causes they are deleterious and a part of cascade of events that can lead to neuron death (Markesbery, 1997). This suggests that therapeutic efforts aimed at removal of free radicals or prevention of their formation may be beneficial in diseases like AD.

In the present study, the results from the passive avoidance behavior and elevated plus maze test show that in the vehicle-treated ICV STZ-treated rats there was impairment of learning and memory as evidenced by significantly reduced retention latencies in passive avoidance behavior and no improvement in retention transfer latency in elevated plus maze. These results are in conformity with other workers who have demonstrated cognitive impairment after ICV STZ in rats (Blokland and Jolles, 1993; Lannert and Hoyer, 1998; Lannert et al., 1998).

However, in both the ICV STZ groups that were chronically treated with melatonin (10 and 20 mg/kg ip), the rats showed significantly increased retention latencies as compared to the STZ (vehicle)-treated animals in the passive avoidance apparatus and significantly shorter transfer latencies on the elevated plus maze as compared to the vehicle-treated ICV STZ animals. The improvement in passive avoidance behavior indicates an improved acquisition and/or retention of memory and the shorter transfer latencies in the elevated plus maze indicate an increased capacity to learn in rats treated with melatonin.

The locomotor activity of sham, vehicle-treated ICV STZ group and both the melatonin-treated ICV STZ rats showed no significant difference. This excludes the possibility that the locomotor activity per se may have contributed to the changes in passive avoidance and elevated plus maze in vehicle-treated ICV STZ rats and the melatonin-treated ICV STZ group.

The results from the estimation of MDA and glutathione indicate that in the rats that were chronically treated with melatonin there was no significant rise in MDA levels after ICV STZ. Further, their was also no decline in the levels of glutathione in the rat brain as compared to the vehicle-treated ICV STZ rats indicating the inhibition of oxidative stress by melatonin in rat brain. In fact, there was a significant increase in the levels of glutathione in the brains of the rats treated with melatonin as compared to the sham rats, this could be due to the inherent antioxidant property of melatonin (Reiter et al., 1997).

MDA is an end product of lipid peroxidation; a measure of free radical generation. The significantly less increase in MDA level in the brain with both the doses of melatonin as compared to the vehicle-treated ICV STZ rats indicates attenuation of lipid peroxidation. Also, there was a simultaneous significant increase in the glutathione levels. Glutathione is an essential tripeptide, an antioxidant found in all animal cells (Kimura et al., 1998). It reacts with the free radicals and can protect cells from singlet oxygen, hydroxyl radical and superoxide radical damage (Sharma et al., 2000). This suggests that antioxidant property of melatonin was responsible for protecting against the oxidative stress, possibly by increasing the endogenous defensive capacity of the brain to combat oxidative stress induced by ICV STZ.

In conclusion, the present study clearly demonstrates that melatonin significantly prevented the cognitive impairment and attenuated the oxidative stress in ICV STZ model in rats. The findings suggest the therapeutic potential of melatonin in age and age related neurodegenerative disorders where oxidative stress and cognitive impairment are involved.

Acknowledgments

Melatonin was a generous gift from Dabur, India.

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