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Antidepressant and Antihyperlipidemic Effects of Melatonin in Streptozotocin-Diabetic Rats

[1]Redouane Rebai, [2]Abdennacer Boudah and [3]Hacène Frih

[1]Department of Biochemistry and Molecular and Cellular Biology,
Faculty of Natural and Life Sciences, University Mentouri Brothers, Constantine, Algeria
[2]Higher National School of Biotechnology, Constantine, Algeria
[3]Department of Biology, Faculty of Science,
University Badji Mokhtar, Annaba, BP 12 El Hadjar, Algeria

Abstract: Diabetes mellitus is considered a serious public health problem, it can cause many complications affecting several organs and functions. The present study was initiated to examine the effect of melatonin on neuropsychological deficits and metabolic disorders which generated by streptozotocin (STZ)-induced diabetes. After confirming the installation of diabetes (48 h), diabetic rats were subjected to a treatment with a dose of melatonin at a rate of (0.5 mg / kg), ip, for 2 weeks and the three groups of experiment underwent the open field test (OFT) and the forced swimming test (FST) model were performed for the evaluation of ambulatory activity and depressive behavior. The last day of the study, blood samples were taken to assess plasma insulin levels and lipid profile in experimental groups. The results show a significant increase in locomotory activity in diabetic rats treated with melatonin, while the latter reduces the immobility time in diabetic group in the FST. Moreover these central effects, melatonin improves lipid profile by reducing serum triglycerides, blood cholesterol levels and raising the HDL cholesterol in periphery. The results of this study suggest that melatonin exerts an antidepressant and antihyperlipidemic effects in diabetes and its complications. Therefore the melatonin could be an alternative treatment for depression to the type 1 diabetic patients.

Key words: Diabetes Mellitus · Depression · Melatonin · Lipid Profile · Streptozotocin

INTRODUCTION

Chronic hyperglycemia is the cause of more or less serious degenerative complications affecting many organs, especially the eyes, kidney, nervous and cardiovascular system. Many studies have proven that there is a known association between diabetes mellitus and behavioral disorders, such as depression and anxiety. In addition to numerous studies have shown the impact of diabetes on the brain structures that results in a change of the monoamine levels of serotonergic, GABAergic and noradrenergic neurons [1] and It has been reported that altered behavior and depression observed in diabetics are strongly correlated with the levels of serotonin and its precursor tryptophan (Trp) in the brain [2,3], these change in serotonergic neurotransmission return to a relative deficiency of insulin secretion in STZ-induced diabetic [4].

Melatonin is a indolamine synthesized by the pineal gland during the dark phase from tryptophan, an essential amino acid provided by food. It plays a major role in regulating the circadian rhythm, acting on the sleep-wake rhythm and influences the mechanisms of reproduction by acting on the hypothalamus and pituitary gland. Besides these neuroendocrine functions, many studies have lent to melatonin antidepressant property [5], it was demonstrated in the forced swim test (FST), a putative model for evaluating the efficacy of antidepressant treatment in rodents [6,7,8]. The anxiolytic effect of melatonin is verified by the open field test (open-field) that is used most often in combination with the (FST) to assess the locomotor activity [9].

Corresponding Author: Redouane Rebai, Department of Biochemistry& Molecular and Cellular Biology,
Faculty of Natural and Life Sciences, University Mentouri Brothers, Constantine, Algeria.
Tel: +21390859187.

On the other hand the studies have focused on the beneficial effects produced by the administration of melatonin, as well as molecule having a regulatory effect on dyslipidemia caused by diabetes mellitus that molecule as improving blood lipid profile by reducing triglycerides, increasing HDL-cholesterol and decreasing the total cholesterol [10, 11].

The objective of this study was to evaluate the effect of melatonin as an antidepressant and anxiolytic in a diabetic rat model on the one hand and on the other to prove its beneficial effect on lipid metabolism disorder induced by injection STZ.

MATERIALS AND METHODS

Biological Material: Wistar female rats (Institut Pasteur, Algiers, Algeria), weighing (160-180 g) were used in this study. The animals are kept in a pet under standard environmental conditions (22 ± 2 ° C, 55 ± 5% humidity and a light cycle of 12 hours day / night) and are subject to a standard diet. All experiments were performed according to a protocol established by the ICLAS (the international council for laboratory animal science).

Chemical Products: Streptozotocin and absolute alcohol were obtained from (Sigma Aldrich, Germany), while melatonin has been provided (Laboratory SOLGAR, France).

Induction of Diabetes and Administration of Melatonin: The rats were randomly divided into two groups at the start of the experiment. The control rats (n = 6) had only a buffer injection. The diabetes was induced by intraperitoneal injection of a single dose of STZ (60 mg / kg) body weight. Just before use, STZ was dissolved in citrate buffer (pH 4.5, 0.1 M). The blood glucose concentration was measured after 2 days of STZ injection, on a sample blood that was taken from the tail vein by using a glucometer (Accu Chek player).

Only rats whose fasting blood glucose> 250 mg / dL were declared diabetics.

Diabetic rats (n = 12) were divided into two subgroups: The first subgroup (n = 6) received a daily dose of melatonin at 0.5 mg / kg (Diabetic+Melatonin) for 14 days.

Melatonin solution was prepared using absolute ethanol that is then diluted in NaCl 0.9%. the final concentration of ethanol is 6% and the volume injected is 0.5 ml. The second sub-group (D) was injected only with the solvent, the injections of the solvent or melatonin were performed at 13: 30h.

Behavioral Study

The Open Field Test: This test is designed to assess emotional reactivity difference in an animal vis à vis a new environment [12] and ambulatory activity which could be altered by administration of drugs or dietary restriction. In addition to information on locomotor activity [13], this test can predict anxiolytic-like activity. The paradigm used is a square enclosure plexiglass 70 cm and 40 cm high. It includes a floor in the form of a square of 10 cm × 10 cm in diameter, it is in two parts: a peripheral part and a central part, each of which is of 35cm. The test takes 5 minutes and involves placing the animal in the central area by evaluating locomotor activity and anxiety of the animal, that is even more pronounced when the rats spent more time in the peripheral zone.

The Forced Swimming Test: The forced swim test is widely used to predict antidepressant-like activity [7].

It consists of placing the rat in an aquarium 40 cm high and 30 cm wide and filled with water at 26 °C over a height of 35 cm, these dimensions allow to ensure that may not use his legs to stand on the surface or escape and thus subjected to forced swimming. The test consists of two separate phases of 24heurs, during the pre-test (the day 13 of the experiment) the rat was placed in the tank for 15 minutes and at the end of this stage the animal is immobile. The second phase lasts for 5 min during which the behavior of the animal was videotaped using a video camera to measure the variables during the test: the immobility time, swimming time and the time of climbing.

Determination of Plasma Insulin and Lipid Parameters: The determination of biochemical parameters was performed at the end of the second week, the blood sample taken from rats fasted and at the retro orbital sinus of the eye, using heparinized capillaries, the blood sample was collected in dry heparin tube and centrifuged at 3500 rpm.

Plasma insulin was assayed by chemiluminescent enzyme immunometric method and serum triglycerides, total cholesterol and HDL-cholesterol were determined by an enzymatic method [14, 15, 16].

Statistical Analysis: The results are expressed as mean ± SEM. Data D were analyzed by one-way ANOVA and Tukey as the post hoc test. Differences are considered significant when $p < 0.05$. Graph Pad Prism 6 for windows version 6.05 was used to do the analysis.

RESULTS

Effect of Melatonin on Behavioral Parameters in the Open Field and Forced Swim

The Open Field Test: Figure 1 shows the variation of parameters of total distances, number of entries in the center and immobility time in the control rats, diabetic control and diabetic treated with melatonin undergoing the open field test (at the twelfth day) of treatment.

The ANOVA1 shows a significant treatment effect of melatonin on the total distance crossed (F = 176.6, R square = 0.9619, $p < 0.0001$). The Tukey test revealed a highly significant increase in the total distance crossed (Fig.A) in diabetic rats treated with melatonin compared to diabetic control: (q = 26.32, p <0.0001).

The ANOVA1 for the center square entries (Fig.B) reported a significant treatment effect of melatonin (F = 28.40, R square = 0.8023, p <0.0001). The Tukey test indicated a significant increase in the number of entries in the center square in diabetic rats treated with melatonin compared to diabetic control: (q = 10.06, p <0.0001).

Regarding immobility time (Fig. 1C), it was observed a very highly significant treatment (F = 76.88, R square = 91.65, p <0.0001). The Tukey test indicated a significant decrease in the immobility time in diabetic rats treated with melatonin compared to diabetic control: (q = 17.03, p <0.0001).

The Forced Swimming Test: ANOVA 1 indicates a very highly significant melatonin effect (F = 27.81, R square = 0.7989, p <0.0001) in the immobility time (Fig 2. A). The Tukey test shows a very highly significant decrease in the immobility time in diabetic rats treated with melatonin compared with diabetic group (q = 10.28, p <0.0001), by cons we have not found significant differences (p □ 0.05) in climbing time (Fig 2.B).

However the ANOVA1 shows a very significant melatonin effect (F =12.87, R square =0.6477, p <0.0007) in swim time (Fig 2. C). The Tukey test revealed in swim time a very highly significant increase of the swim time in the diabetic group undergoing treatment of melatonin compared to the untreated diabetic group (q= 6.75, p <0.0001).

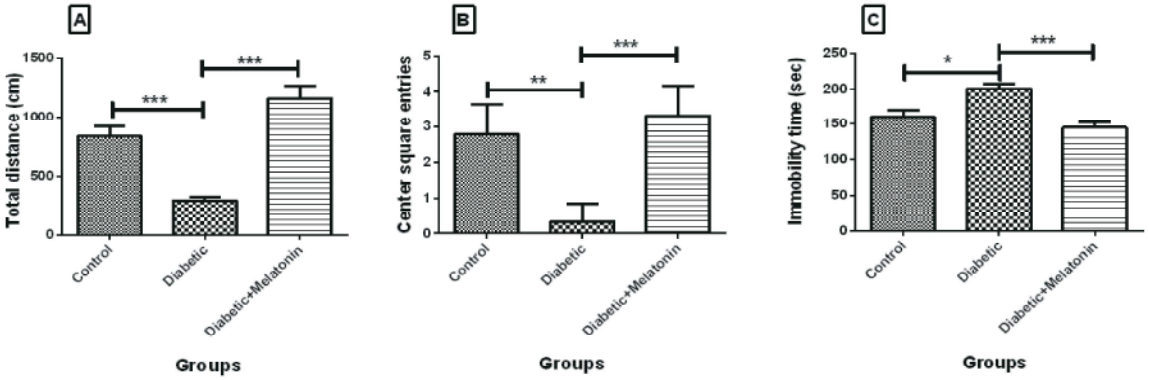

Fig. 1: Behavioral parameters in the open field test in diabetic rats treated and untreated with the melatonin. A: Total distance, B: Center square entries, C: Immobility time. *p<0.05, **p<0.001, ***p<0.0001.

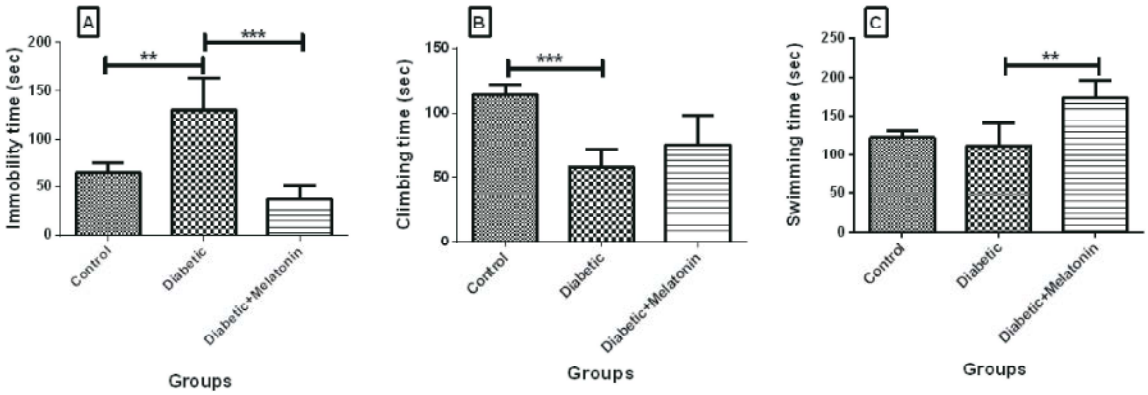

Fig. 2: Behavioral parameters in the forced swimming test in diabetic rats treated and untreated with melatonin. A: Immobility time, B:Climbing time, C: Swimming time. **p<0.001, ***p<0.0001.

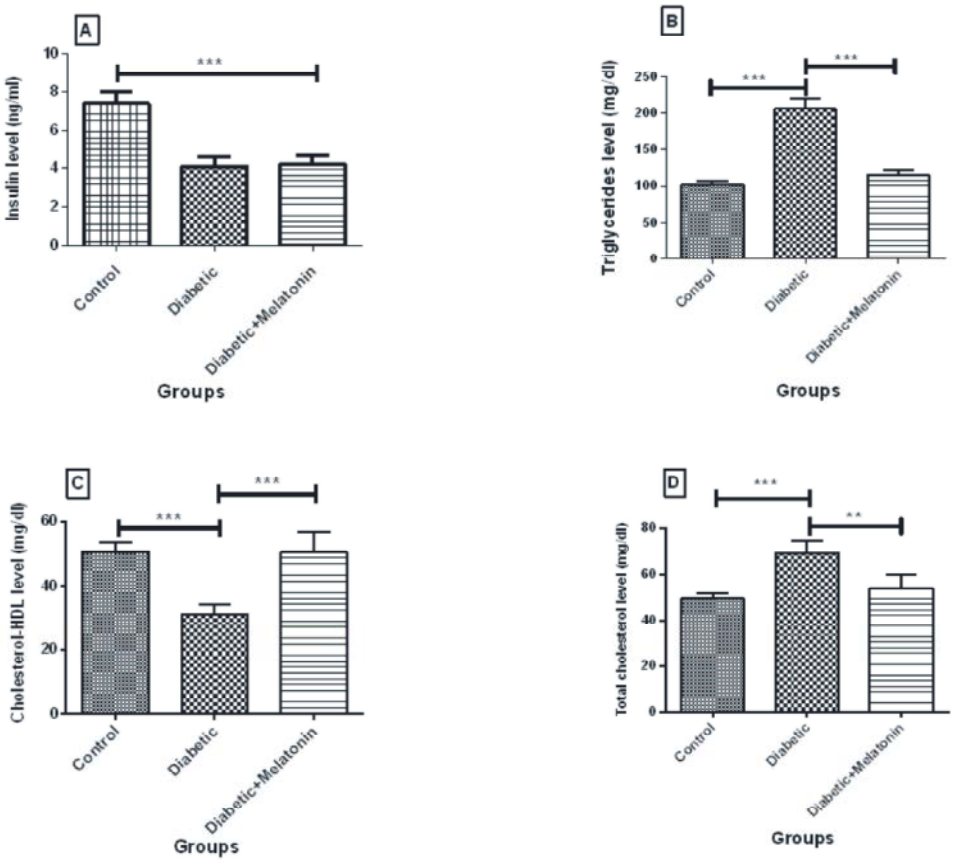

Fig. 3: Plasma insulin and lipid profile parameters in *Wistar* rats. A: Plasma insulin, B: Triglycerides, C: HDL-cholesterol, D: Total cholesterol in control group, diabetic and diabetic treated with melatonin. ** p <0.001, *** p <0.0001.

Effect of Melatonin on Plasma Insulin and Lipid Parameters: ANOVA1 shows a highly significant difference (p<0.0001) between the three groups, but the Tukey test does not show a significant effect of melatonin on insulin levels in diabetic group treated with melatonin compared with the control diabetic.

For lipid profile, ANOVA1 indicated a very significant melatonin effect (F = 184.9, R square = 0.9635, p <0.0001) in triglyceride level. The Tukey test shows a very highly significant decrease of triglyceride level in diabetic rats treated with melatonin compared with diabetic group (q= 22.23, p <0.0001), while we recorded a highly significant elevation in HDL-cholesterol in rats treated with melatonin compared to the diabetic group (q = 10.39, p <0.0001).

However, a very highly significant effect of melatonin in total cholesterol was indicated by ANOVA1(F = 26.56, R square =0.7914, p <0.0001). The Tukey test revealed a very highly significant decrease in total cholesterol was noted in the treated diabetic compared with untreated diabetic (q = 7.796, p <0.0001).

DISCUSSION

Diabetes mellitus is a metabolic dysfunction characterized by chronic hyperglycemia due to insulin deficiency. This disorder in the glucose and lipid metabolism is often accompanied by mood disorders and deficits in locomotor activity [17, 18].

In this study we examined the melatonin as a molecule with antidepressant and anti-dyslipidemic effects in streptozotocin- diabetic rats.

Our results (Fig 1) indicate that the administration of melatonin in diabetic rats at in open field test has increased very significantly locomotor activity, this indicates anxiolytic activity of melatonin. Similarly [19, 20] have reported that treatment with melatonin induced an increase in level of exploration in animals and decreased the duration of immobility. The present study demonstrated that treatment with melatonin in diabetic rats has actually decreased the immobility time in the FST, this indicates an antidepressant-like effect of this drug [21, 22]. These behavioral changes back to the interaction

of the hormone with the serotonergic system in which the reduction in immobility time is in favor of an increase in the swimming time [23], which is consistent with our results.

In addition, it was reported that the antidepressant effect was generated by the interaction of melatonin with 5-HT2A receptors that are expressed at high density in depressed patients [24]. At this point, melatonin would act as an antagonist of these receptors [25]. At the periphery, administration of melatonin causes no significant change in insulin treated diabetics compared with diabetic controls. This is probably due to the destructive effect of STZ on pancreatic beta islets. Similar results are consistent with those provided by [26]. Our study demonstrated the lipid-lowering effect of melatonin, while installation of diabetes caused a large elevated triglycerides, total cholesterol and lower HDL cholesterol, however, administration of melatonin has standardized this dyslipidemia in diabetic group.

In conclusion, our results suggest melatonin seems to play a crucial role in the prevention of diabetes mellitus complications first by its effects at the central level as an antidepressant, but also as a drug having anti-hyperlipidemic activity in periphery when metabolic disorders which are observed in type 1 diabetic patients. It could be suggested as an alternative treatment for depression and lipid disorders associated with diabetes mellitus.

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