

# Blood pressure reducing effects of piromelatine and melatonin in spontaneously hypertensive rats

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**Abstract. – BACKGROUND:** Recently, widespread interest has grown regarding melatonin treatment of hypertension including its cardioprotective effects. Studies in rodents indicate that melatonin plays a role in the pathogenesis of hypertension in rats with metabolic syndrome.

Piromelatine, a melatonin agonist, serotonin 5-HT<sub>1A</sub> and 5-HT<sub>1D</sub> agonist and serotonin 5-HT<sub>2B</sub> antagonist is a multimodal agent with sleep promoting, anti-diabetic, analgesic, anti-neurodegenerative, anxiolytic and antidepressant potential, currently in development for the treatment of insomnia.

**AIM:** In this report we assessed the effects of piromelatine and melatonin treatment on blood pressure in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats.

**MATERIALS AND METHODS:** Five groups of 12-wk-old rats (10/group) were treated for 5 weeks with a vehicle, piromelatine (5, 15 and 50 mg/kg BW) and melatonin (10 mg/kg BW) and an age-matched WKY control group. Systolic blood pressure (tail-cuff method) was measured weekly at 9:00 a.m. and at 9:00 p.m. The rats body weight, plasma glucose, insulin, triglyceride, adiponectin, total cholesterol, HDL and LDL/VLDL cholesterol were also measured.

**RESULTS:** Our results showed that both piromelatine and melatonin reduced SHR blood pressure significantly both during the morning and the evening. Piromelatine, but not melatonin, also reduced SHR body weight gain and both significantly decreased plasma glucose and insulin levels and increased adiponectin levels.

**CONCLUSIONS:** Piromelatine, similar to melatonin, has an antihypertensive effect and also attenuates body weight, improves metabolic profiles and might be useful in the treatment of hypertension and the metabolic syndrome.

*Key Words:*

Piromelatine, Melatonin, Hypertension, Spontaneously hypertensive rats, Metabolic diseases.

## Introduction

The pineal hormone melatonin is secreted with a marked circadian rhythm. The endogenous rhythm of secretion is generated by the suprachiasmatic nuclei, entrained to the light/dark cycle and conveys information concerning the daily cycle to body physiology functions such as sleep, immune system and glucose regulation<sup>1</sup>. Recent data indicate that impaired melatonin production is involved in several cardiovascular pathologies including hypertension and ischemic heart disease<sup>2</sup>. In addition, some reported that melatonin can reduce increased blood pressure both in hypertensive humans and animals<sup>3</sup>. The blood pressure lowering effect of melatonin was demonstrated in healthy women taking contraceptive pills, postmenopausal women with hormone replacement therapy, healthy men and hypertension patients<sup>4</sup>.

Diminished melatonin production at night is consistently reported in hypertensive patients with nondipping blood pressure and in patients with coronary heart disease. Pinealectomy, which was associated with decreased melatonin production, caused vasoconstriction and temporary hypertension in adult rats while continuous light (24 hours/day), which prevented the nocturnal

rise of melatonin plasma levels, also resulted in suppression of circadian heart rate and blood pressure variability<sup>1</sup>. Melatonin has been shown to reduce blood pressure, oxidative load and to increase nitric oxide bioavailability in spontaneously hypertensive rats (SHR). Blood pressure decreased after 6 weeks of melatonin treatment (10 mg/kg) of SHR, which was associated with a reduction of interstitial renal tissue inflammation, decreased oxidative stress and attenuation of expression of nuclear factor kappa B in the kidney<sup>5</sup>.

In the same model of hypertension, melatonin, in addition to lowering mean arterial pressure and causing a heart rate reduction, restored plasma norepinephrine concentration and the proportion of  $\beta_1/\beta_2$  receptors in the heart, enhanced maximal relaxation of mesenteric arteries and improved baroreflex responses, which relate to its antioxidant effects. Acute administration of melatonin lowered blood pressure and reduced norepinephrine blood levels in SHR<sup>6-8</sup>.

*In vitro*, melatonin attenuated constriction of aortic ring in SHR were demonstrated to inhibit phospholipase-C cascade independently on melatonin receptor or  $\alpha_1$ -adrenoceptor blockade and prevent excess oxidative load related with the antioxidant enzyme superoxide dismutase<sup>9</sup>.

Melatonin secretion decreased in fructose fed rats, an animal model of the metabolic syndrome, that developed hypertension and the administration of melatonin blunted this blood pressure rise<sup>10</sup>.

The plasma half-life of melatonin is only 35 to 50 minutes. Attempts have been made to remedy this problem via a controlled-release formulation of melatonin and by developing various indolic and non-indolic melatonergic agonists.

Piromelatine (former name is Neu-P11) is a melatonin agonist, serotonin 5-HT<sub>1A</sub> and 5-HT<sub>1D</sub> agonist and serotonin 5-HT<sub>2B</sub> antagonist. Piromelatine is a multimodal agent with sleep promoting, anti-diabetic, analgesic, anti-neurodegenerative, anxiolytic and antidepressant potential, currently in development for the treatment of insomnia.

Recent studies have shown that piromelatine promoted sleep<sup>11</sup>, exerts antidepressant and anxiolytic activities in rodent models<sup>12</sup> and inhibited weight gain and improved insulin sensitivity in high-fat/high-sucrose-fed rats<sup>13</sup>. In the present study we investigated the potential effects of piromelatine and melatonin on blood pressure and metabolic profiles of SHR with established hypertension.

## Materials and Methods

### Materials

Piromelatine (C<sub>17</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>) and melatonin were provided by Neurim Pharmaceuticals Ltd. (Tel-Aviv, Israel), both were dissolved in 1% dimethylsulfoxide (DMSO)/water solution.

### Animals and Treatment

12-wks-old male spontaneously hypertensive rats (SHR) were randomly divided into 5 groups (n=10 in each group): control SHR group, piromelatine (5 mg/kg/day) treated SHR group, piromelatine (15 mg/kg/day) treated SHR group, piromelatine (50 mg/kg/day) treated SHR group and melatonin (10 mg/kg/day) treated SHR group. In addition age-matched Wistar-Kyoto rats (WKY) were divided into 5 groups (n=10 in each group) with the same treatment as the SHR to serve as control. Both drugs were given with intraperitoneal injection in 18:00 every day. All animals were housed at a temperature of 23±1°C in individual cages and freely fed a regular pellet diet ad libitum. All rats were subjected to alternate 12 h periods of dark and light (lights on 6:00 a.m.-6:00 p.m.).

### Blood Pressure Measurements

Systolic blood pressure (SBP) was determined weekly in conscious restrained rats by tail-cuff plethysmography at 9:00 a.m. and at 9:00 p.m.. Before the beginning of the experiments, rats were conditioned three to four times to the procedure and when blood pressure was determined, the reported values represented the mean of three to four separate determinations. After five weeks of treatment, blood pressure was determined by direct puncture of the carotid. Body weights (BW) and food consumption were measured once a week.

### Biochemical Parameters

Plasma was separated by centrifugation (12,000 rpm, 4 min) and stored at -80°C. Glucose was determined by a chemical colorimetric method (BioAssay Systems, Hayward, CA, USA). Plasma insulin and adiponectin were determined by ELISA (Biocompare, San Francisco, CA, USA). Plasma triglycerides (TG), total Cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein/very low-density lipoprotein (LDL/VLDL) cholesterol were determined by commercially available enzymatic methods (BioAssay Systems, Hayward, CA, USA).

**Statistical Analysis**

All quantitative data are expressed as mean ± SD. One-way ANOVA test was used to analyze the significance of differences between mean values. A level of  $p < 0.05$  was considered statistically significant.

**Results**

**Blood Pressure**

After five weeks of treatment, the systolic blood pressure at 9:00 a.m. and 9:00 p.m. of WKY rats was  $126.8 \pm 5.7$  mmHg and  $122.8 \pm 4.2$  mmHg, respectively, and both piromelatine and melatonin treatments had no markedly effect on the SBP of WKY rats. Blood pressure of 12-week-old SHR was significantly higher in comparison with normotensive WKY rats in accordance with literature data<sup>14</sup>. SHR developed higher hypertension by 12-18 weeks of age, reaching  $181.8 \pm 6.2$  mm Hg at 9:00 a.m. and  $175.4 \pm 5.1$  at 9:00 p.m. ( $p < 0.05$ ) in comparison to control WKY rats (Table I). The systolic blood pressure in 18-week-old piromelatine and melatonin-treated SHR was significantly lower than untreated rats noted as early as the second (p.m. measurement) or third (a.m. measurement) week of treatment (Table I). The systolic blood pressure in 18-week-old piromelatine and melatonin-treated SHR decreased after five weeks of treatment with 5 mg/kg piromelatine ( $172.3 \pm 5.9$  mm Hg,  $p < 0.05$ ), 15 mg/kg piromelatine ( $161.8 \pm 5.5$  mm Hg,  $p < 0.05$ ), 50 mg/kg piromelatine ( $156.3 \pm 4.2$  mmHg,  $p < 0.05$ ) and melatonin 10 mg/kg treatment ( $151.9 \pm 4.5$  mmHg,  $p < 0.05$ ) at 9 a.m. in comparison to untreated SHR. In addition, treatment with 15 mg/kg, 50 mg/kg piromelatine and 10 mg/kg melatonin caused a significant reduction of nocturnal blood pressure at 9:00 p.m. ( $145.7 \pm 3.5$  mm Hg,  $140.7 \pm 5.8$  mm Hg and  $143.5 \pm 4.4$  mm Hg, respectively,  $p < 0.05$ ) as compared with untreated SHR ( $175.4 \pm 5.1$  mm Hg).

**Body Weight and Food Consumption**

Body weight of SHR and WKY rats were increased by 18.3% and 30%, respectively, during the 5 weeks of the study. Treatment with 5-50 mg/kg piromelatine significantly decreased the body weight of SHR compared to untreated SHR ( $p < 0.05$ ). However melatonin treatment had no significant effect. Both piromelatine and melatonin did not affect the body weight of WKY rats (Figure 1a and b). Food consumption was unchanged in all groups of rats during the study.

**Table I.** Effect of 5 weeks administration of piromelatine and melatonin on systolic blood pressure and body weight (10 per group).

Time of SBP measurement	Treatment period (week)	SHR (mmHg)	SHR+ 5 mg/kg piromelatine (mmHg)	SHR+ 15 mg/kg piromelatine (mmHg)	SHR+ 50 mg/kg piromelatine (mmHg)	SHR+ 10 mg/kg melatonin (mmHg)	WKY (mmHg)	WKY+ 5 mg/kg piromelatine (mmHg)	WKY+ 15 mg/kg piromelatine (mmHg)	WKY+ 50 mg/kg piromelatine (mmHg)	WKY+ 10 mg/kg melatonin (mmHg)
9 am	1	169.2 ± 4.6	164.2 ± 4.8	165.5 ± 3.6	164.1 ± 4.5	164.0 ± 4.4	124.9 ± 3.5 <sup>a</sup>	125.1 ± 4.5 <sup>a</sup>	122.2 ± 3.4 <sup>a</sup>	125.1 ± 3.3 <sup>a</sup>	128.3 ± 5.1 <sup>a</sup>
9 am	2	172.4 ± 4.1	166.2 ± 5.2	163.6 ± 4.4	166.3 ± 4.9	158.4 ± 3.7 <sup>b</sup>	123.7 ± 3.7 <sup>a</sup>	127.2 ± 4.8 <sup>a</sup>	124.1 ± 4.8 <sup>a</sup>	127.3 ± 2.9 <sup>a</sup>	127.2 ± 3.8 <sup>a</sup>
9 am	3	177.1 ± 7.4	169.8 ± 4.9	159.2 ± 3.7 <sup>b</sup>	162.6 ± 4.6 <sup>b</sup>	157.8 ± 3.5 <sup>b</sup>	123.2 ± 3.5 <sup>a</sup>	123.9 ± 4.1 <sup>a</sup>	122.8 ± 3.9 <sup>a</sup>	122.9 ± 3.6 <sup>a</sup>	123.4 ± 3.5 <sup>a</sup>
9 am	4	178.9 ± 5.5	168.1 ± 5.1 <sup>b</sup>	161.9 ± 5.6 <sup>b</sup>	160.9 ± 4.4 <sup>b</sup>	155.3 ± 4.1 <sup>b</sup>	122.8 ± 2.4 <sup>a</sup>	122.7 ± 3.5 <sup>a</sup>	125.1 ± 3.8 <sup>a</sup>	125.3 ± 4.2	126.8 ± 2.9 <sup>a</sup>
9 am	5	181.8 ± 6.2	172.3 ± 5.9 <sup>b</sup>	161.8 ± 5.5 <sup>b</sup>	156.3 ± 4.2 <sup>b</sup>	151.9 ± 4.5 <sup>b</sup>	126.8 ± 5.7 <sup>a</sup>	126.5 ± 4.1 <sup>a</sup>	124.1 ± 4.6 <sup>a</sup>	122.1 ± 4.2 <sup>a</sup>	124.3 ± 3.9 <sup>a</sup>
9 pm	1	158.7 ± 5.7	158.2 ± 4.4	158.4 ± 3.9	155.2 ± 4.1	155.1 ± 3.8	117.4 ± 3.9 <sup>a</sup>	124.2 ± 2.9 <sup>a</sup>	119.5 ± 3.5 <sup>a</sup>	120.5 ± 2.9 <sup>a</sup>	121.5 ± 3.5 <sup>a</sup>
9 pm	2	162.5 ± 6.3	157.8 ± 5.1	151.5 ± 5.2 <sup>b</sup>	152 ± 3.7 <sup>b</sup>	152.5 ± 4.7 <sup>b</sup>	121.5 ± 3.2 <sup>a</sup>	120.8 ± 3.3 <sup>a</sup>	122.6 ± 2.7 <sup>a</sup>	123.1 ± 3.4 <sup>a</sup>	120.4 ± 3.1 <sup>a</sup>
9 pm	3	168.1 ± 6.8	158.3 ± 6.2	144.6 ± 4.8 <sup>b</sup>	144.5 ± 5.2 <sup>b</sup>	145.6 ± 4.1 <sup>b</sup>	123.5 ± 2.4 <sup>a</sup>	122.9 ± 2.8 <sup>a</sup>	124.2 ± 3.4 <sup>a</sup>	124.1 ± 2.5 <sup>a</sup>	118.9 ± 2.4 <sup>a</sup>
9 pm	4	170.9 ± 7.1	156.5 ± 5.2 <sup>b</sup>	146.2 ± 3.2 <sup>b</sup>	142.8 ± 3.5 <sup>b</sup>	145.9 ± 3.5 <sup>b</sup>	120.8 ± 3.4 <sup>a</sup>	121.4 ± 4.4 <sup>a</sup>	118.8 ± 2.9 <sup>a</sup>	120.5 ± 3.4 <sup>a</sup>	120.2 ± 3.8 <sup>a</sup>
9 pm	5	175.4 ± 5.1	160.8 ± 5.2	145.7 ± 3.5 <sup>b</sup>	140.7 ± 5.8 <sup>b</sup>	143.5 ± 4.4 <sup>b</sup>	122.8 ± 4.2 <sup>a</sup>	120.9 ± 3.9 <sup>a</sup>	115.6 ± 4.1 <sup>a</sup>	118.7 ± 3.8 <sup>a</sup>	119.2 ± 4.1 <sup>a</sup>

Data are means ± SD, Significant differences ( $p < 0.05$ ): compared to corresponding SHR, bcompared to untreated SHR. Systolic blood pressure (SBP) was determined in conscious restrained rats by tail-cuff plethysmography (weeks 1-4) and by direct puncture of the carotid (week 5).

### Plasma Glucose and Insulin

As shown in Figure 2a, plasma glucose concentration was significantly higher in the SHR compared with WKY rats ( $p < 0.05$ ; WKY vs. corresponding SHR). After 5 weeks of treatments with 5 mg/kg, 15 mg/kg and 50 mg/kg piromelatine, plasma glucose was decreased significantly by 27.3%, 34.5% and 61.5%, respectively, in the treated SHR ( $p < 0.05$ ) compared to untreated SHR. The plasma level of insulin in SHR was significantly higher than that in WKY rats (Figure 2b). The treatment with 5 mg/kg, 15 mg/kg and 50 mg/kg of piromelatine or 10 mg/kg melatonin resulted in a significant decrease in plasma level of insulin in SHR (34.5%, 56.9%, 67.2% and 51.5%, respectively,  $p < 0.05$ ) compared to untreated SHR.

In the WKY rats, 15 mg/kg and 50 mg/kg of piromelatine significantly decreased the plasma level of glucose while 5 mg/kg and 50 mg/kg of piromelatine and 10 mg/kg of melatonin significantly decreased insulin plasma levels compared to untreated WKY rats (Figure 1b).

### Effects of Treatments on Rat's Lipid Metabolism

Figure 3 shows the concentrations of triglycerides. The level of triglycerides in SHR was significantly higher than that in WKY ( $p < 0.05$ ; WKY vs. corresponding SHR, Figure 3). After 5

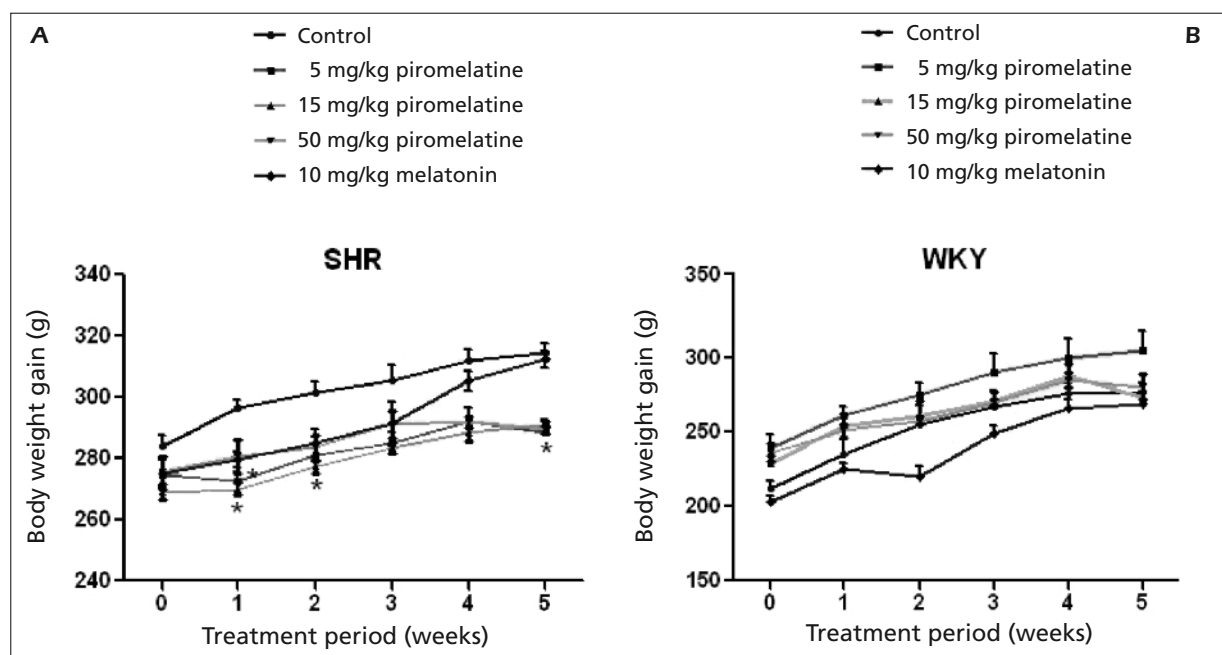
weeks treatment with melatonin the triglycerides level had been significantly suppressed compared with SHR ( $0.66 \pm 0.06$  mmol/L vs.  $0.75 \pm 0.11$  mmol/L), but piromelatine had no effect ( $p > 0.05$ , vs. SHR). There were no significant differences in plasma levels of TC, LDL-C and HDL-C between SHR and WKY despite of treatment with piromelatine or melatonin (data not shown).

### Effects of Treatments on Plasma Adiponectin

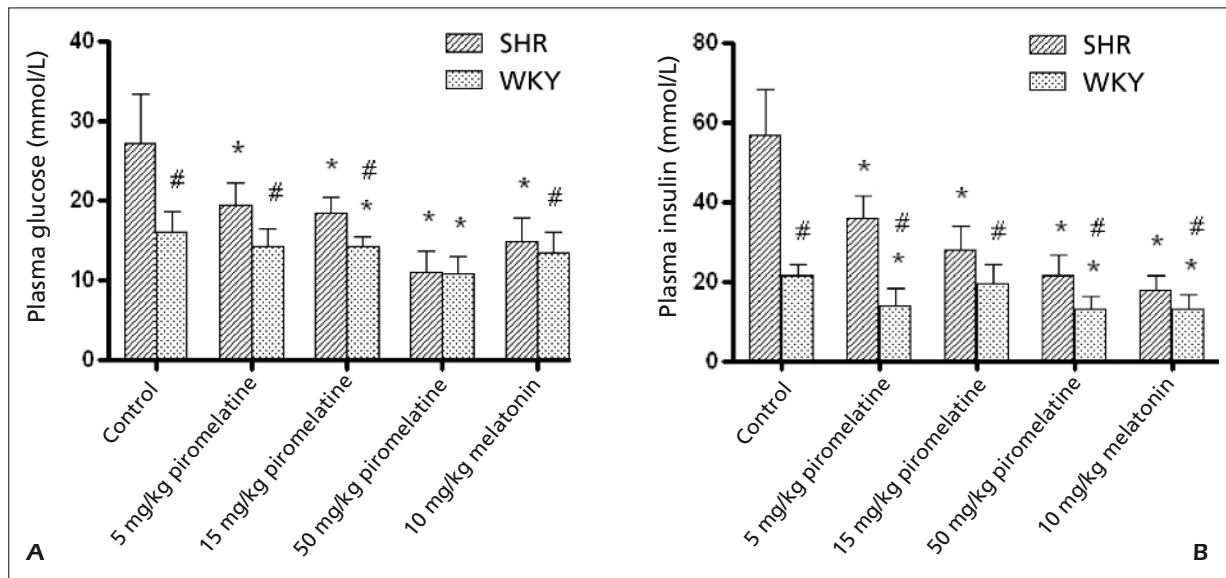
As shown in Figure 4, the plasma level of adiponectin in SHR was significantly lower than in WKY ( $p < 0.05$ ). Treatment with either 15 mg/kg and 50 mg/kg piromelatine or 10 mg/kg melatonin could markedly increase the plasma level of adiponectin in SHR ( $p < 0.05$ ) and the plasma level of adiponectin of SHR administrated with 15 mg/kg and 50 mg/kg piromelatine even reached a level comparable to the WKY rats.

## Discussion

Hypertension, obesity, insulin resistance and their associated vascular complications are reaching epidemic proportion worldwide<sup>15</sup>. Here, we demonstrate that piromelatine like melatonin markedly reduced hypertension, decreased weight gain and im-



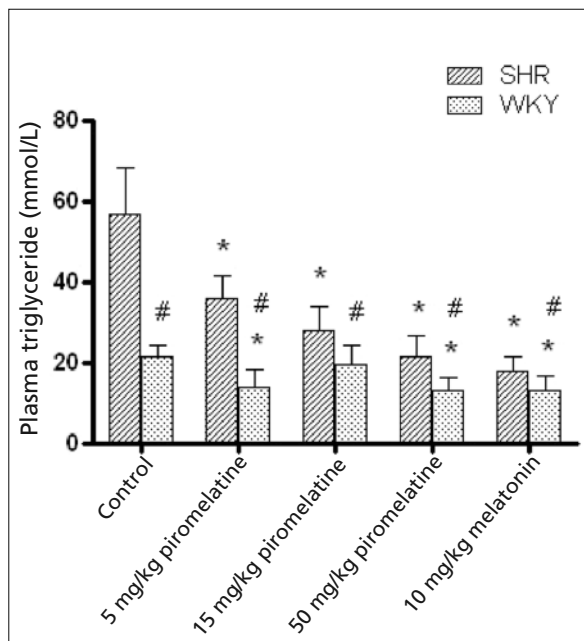
**Figure 1.** The effect of piromelatine on body weight of SHR and WKY rats (**A**) SHR body weight. **B**, WKY body weight. Data are expressed as means  $\pm$  SD ( $n=10$ ), \* $p < 0.05$  treated vs. untreated rats of the same genotype.



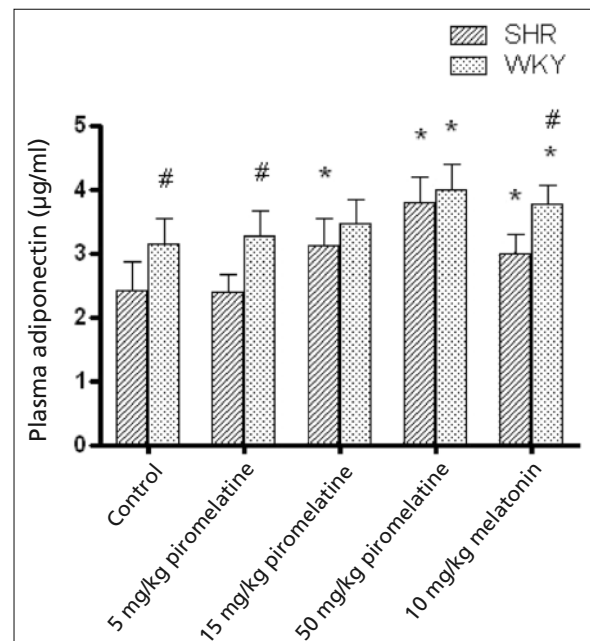
**Figure 2.** Plasma glucose and insulin concentrations by piromelatine and melatonin treatments. **A**, Plasma glucose. **B**, Plasma insulin. Data are expressed as means  $\pm$  SD (n=10), \* $p$  < 0.05 treated vs. untreated rats of the same genotype, # $p$  < 0.05 WKY vs. corresponding SHR.

proved glucose and insulin homeostasis in the SHR rat model of hypertension. The endogenous production of melatonin is regulated by a circadian rhythm, and timing of melatonin administration may be important<sup>16</sup>. In humans, melatonin secretion

increases soon after the onset of darkness, peaks in the middle of the night (between 2 and 4 a.m.) and gradually falls during the second half of the night. In this study, all drugs were injected at 6 p.m., the onset of the dark period of the rats.



**Figure 3.** The effect of piromelatine and melatonin on triglyceride concentrations. Data are expressed as means  $\pm$  SD (n=10), \* $p$  < 0.05 treated vs. untreated rats of the same genotype, # $p$  < 0.05 WKY vs. corresponding SHR.



**Figure 4.** The effect of piromelatine and melatonin on plasma adiponectin. Data are expressed as means  $\pm$  SD (n=10), \* $p$  < 0.05 treated vs. untreated rats of the same genotype, # $p$  < 0.05 WKY vs. corresponding SHR.

The major new finding of our study was that a 5-wks treatment with both piromelatine and melatonin were able to significantly reduce both morning and evening blood pressure in SHR. Melatonin has been shown to reduce systolic blood pressure in Sprague-Dawley rats fed with high fructose<sup>17</sup>. The hypotensive effect of piromelatine might be mediated via melatonin receptors. The involvement of melatonin receptors in regulation of blood pressure was further supported by the finding that the hypotensive effect of microinjection of melatonin into specific brain structures was almost completely prevented by luzindole, an antagonist of the melatonin MT1/MT2 receptors<sup>18</sup>.

In our previous work piromelatine effectively decreased body weight gain in obese rats established using high-fat/high-sucrose-fed for 5 months<sup>13</sup>. In addition, daily administration of melatonin (0.2 microg/mL) via drinking water decreased weight in 10 months-old but not in 3-months-old rats<sup>19</sup>. It is notable that in ovariectomized (Ovx) rats melatonin reduced food intake and partially prevented the increase of body weight. Furthermore, melatonin affected body weight in a model closer to that observed in Western populations, i.e. Sprague Dawley rats fed with a high-fat diet and prevented diet-induced decrease of insulin sensitivity<sup>20</sup>. Daily melatonin administration to middle-aged male rats was also shown to decrease body weight, intraabdominal adiposity, and plasma insulin and leptin concentrations while increasing physical activity, body temperature, and basal plasma corticosterone levels<sup>21</sup>. These results suggest that the decrease in endogenous melatonin secretion may alter energy regulation in middle age, resulting in detrimental metabolic consequences.

Melatonin has no effect in rats on normal body weight but it alters body fat mass which is elevated during aging or in genetically obese rats<sup>20</sup>.

In this study piromelatine, but not melatonin, decreased body weight gain in SHR with no influence on food intake. We hypothesized that higher body temperature and/or higher locomotor activity in piromelatine treated rats might result in a decreased body weight. This hypothesis is consistent with the results of our previous investigation on the effects of piromelatine on body weight gain in obese rats<sup>13</sup>.

In models with altered body weight, such as middle-aged, obese rats and high-fat/high-sucrose-fed rats melatonin may improve insulin sensitivity<sup>13,22</sup>. It has been documented that pinealectomy causes glucose intolerance and de-

creases daily secretion of insulin stimulated by glucose intake and insulin secretion by isolated pancreatic islets<sup>23,24</sup>. Melatonin was effective in treatment of metabolic syndrome induced by high caloric diet or by antipsychotic drugs. Melatonin decreased plasma glucose (13%), leptin (28%) and triglyceride (28%) levels but had no effect on plasma insulin level in Sprague Dawley rats with diet-induced obesity<sup>21</sup>. Furthermore, in pinealectomized high-fat diet rats, body weight gain and feed efficiency were increased 4-wks after surgery. Adipose tissue weight, insulinemia, and glycemia had a tendency to increase. Treatment with melatonin prevented in part these changes. These data demonstrate that melatonin may act as a regulator of body weight in a model of obesity and may prevent some of the side effects on glucose homeostasis such as decreased insulin sensitivity<sup>21</sup>.

Insulin resistance (IR) plays a key role in the pathogenesis of the insulin resistance syndrome<sup>25</sup>. IR caused by hyperglycemia and dyslipidemia seems to be linked to many features of the metabolic diseases. The role of central nervous system in regulating plasma glucose has been demonstrated in supra-chiasmatic nuclei (SCN)-lesion studies. Without a functioning SCN, cortisol and glucose do not rise before the beginning of the active period (morning arousal)<sup>26,27</sup>. Additionally, damaging the SCN also eliminates the circadian physiology of the pineal gland and loss of the pineal melatonin alters glucose homeostasis in pinealectomized rats<sup>28</sup>.

We also observed that piromelatine as well as melatonin improved insulin intolerance in SHR as verified by a significant reduction of glucose and insulin levels with no change in total cholesterol, LDL-C and HDL-C levels. These results were coinciding with the findings of our previous report on the effects of piromelatine on body weight gain in obese rats<sup>13</sup>.

Accumulating evidence indicate that plasma adiponectin is lower (hypoadiponectinemia) in patients with diabetes, hypertension, and dyslipidemia than BMI-matched controls, indicating that hypoadiponectinemia is associated with an increased prevalence of coronary risk factors<sup>29</sup>. Using adiponectin to prevent cardiovascular disease might be a therapeutic strategy. In the current work, piromelatine significantly increased the plasma adiponectin levels. So we can speculate that piromelatine may be a candidate drug to prevent and treat cardiovascular diseases associated with the metabolic syndrome.

To our knowledge, this is the first evidence of a positive effect of piromelatine on overweight and glucose levels in SHR, supporting the proposition that piromelatine like melatonin may ameliorate hypertension, overweight and insulin resistance in humans.

Since these benefits occurred in young-adult rats, before aging induced metabolic and vascular complications, piromelatine might help to prevent cardiovascular disease associated with hypertension and insulin resistance.

### Conclusions

The results of our study suggest that piromelatine, a novel melatonin agonist, possess the effects of melatonin in attenuating the development of hypertension in adult SHR. We have demonstrated that piromelatine treatment may not only reduce body weight and blood pressure but also improve glucose homeostasis and may help to prevent vascular complications in hypertensive/insulin-resistant patients.

### Acknowledgements

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### Conflict of Interest

Moshe Laudon is an employee of Neurim Pharmaceuticals Ltd. All other Authors declare that they have no conflicts of interest.

### References

- 1) BRZEZINSKI A. Melatonin in humans. *N Engl J Med* 1997; 336: 186-195.
- 2) PAULIS L, ŠIMKO F. Blood pressure modulation and cardiovascular protection by melatonin: potential mechanisms behind. *Physiol Res* 2007; 56: 671-684.
- 3) ŠIMKO F, PAULIS L. Melatonin as a potential antihypertensive treatment. *J Pineal Res* 2007; 42: 319-322.
- 4) ARANGINO S, CAGNACCI A, ANGIOLUCCI M, VACCA AM, LONGU G, VOLPE A, MELIS GB. Effects of melatonin on vascular reactivity, catecholamine levels, and blood pressure in healthy men. *Am J Cardiol* 1999; 83: 1417-1419.
- 5) NAVA M, QUIROZ Y, VAZIRI N, RODRIGUEZ-ITURBE B. Melatonin reduces renal interstitial inflammation and improves hypertension in spontaneously hypertensive rats. *Am J Physiol Renal Physiol* 2003; 284: 447-454.
- 6) GIROUARD H, CHULAK C, LEJOSSEC M, LAMONTAGNE D, DE CHAMPLAIN J. Chronic antioxidant treatment improves sympathetic functions and beta-adrenergic pathway in the spontaneously hypertensive rats. *J Hypertens* 2003; 21:179-188.
- 7) GIROUARD H, CHULAK C, LEJOSSEC M, LAMONTAGNE D, DE CHAMPLAIN J. Vasorelaxant effects of the chronic treatment with melatonin on mesenteric artery and aorta of spontaneously hypertensive rats. *J Hypertens* 2001; 19: 1369-1377.
- 8) GIROUARD H, DENAULT C, CHULAK C, DE CHAMPLAIN J. Treatment by N-acetylcysteine and melatonin increase cardiac baroreflex and improves antioxidant reserve. *Am J Hypertens* 2004; 17: 947-954.
- 9) K-LAFLAMME A, WU L, FOUCART S, DE CHAMPLAIN J. Impaired basal sympathetic tone and alpha1-adrenergic responsiveness in association with the hypotensive effect of melatonin in spontaneously hypertensive rats. *Am J Hypertens* 1998; 11: 219-229.
- 10) LEIBOWITZ A, PELEG E, SHARABI Y, SHABTAI Z, SHAMISS A, GROSSMAN E. The role of melatonin in the pathogenesis of hypertension in rats with metabolic syndrome. *Am J Hypertens* 2008; 21: 348-351.
- 11) LAUDON M, URADE Y, HUANG Z. Piromelatine. A novel melatonin agonist: effects on sleep and EEG power spectra in rats. *Sleep* 2008; 31(Suppl): Abst 34.
- 12) TIAN SW, LAUDON M, HAN L, JUN G, FU-LIAN H, YU-FENG Y, HAI-FENG D. Antidepressant and anxiolytic effects of the novel melatonin agonist Neu-P11 in rodent models. *Acta Pharmacol Sinica* 2010; 31: 775-783.
- 13) SHE M, DENG X, GUO Z, LAUDON M, HU Z, LIAO D, HU X, LUO Y, SHEN Q, SU Z, YIN W. Neu-P11, a novel melatonin agonist, inhibits weight gain and improves insulin sensitivity in high-fat/high-sucrose-fed rats. *Pharmacol Res* 2009; 59: 248-253.
- 14) AITMAN TJ, PRAVENEK M, KURTZ TW, SCOTT J. The spontaneously hypertensive rat: a model to dissect cellular defects in glucose and fatty acid metabolism. *Pathol Biol (Paris)* 1998; 46: 693-694.
- 15) NDUHIRABANDI F, DU TOIT EF, LOCHNER A. Melatonin and the metabolic syndrome: a tool for effective therapy in obesity-associated abnormalities? *Acta Physiol (Oxf)* 2012.
- 16) CAJOCHEN C, KRÄUCHI K, WIRZ-JUSTICE A. Role of melatonin in the regulation of human circadian rhythms and sleep. *J Neuroendocrinol* 2003; 15: 432-437.
- 17) LEIBOWITZ A, PELEG E, SHARABI Y, SHABTAI Z, SHAMISS A, GROSSMAN E. The role of melatonin in the pathogenesis of hypertension in rats with metabolic syndrome. *Am J Hypertens* 2008; 21: 348-351.
- 18) NAKAHARA K, KAWANO T, SHIOTA K, MURAKAMI N. Effects of microinjection of melatonin into various brain regions of Japanese quail on locomotor activity and body temperature. *Neurosci Lett* 2003; 345: 117-120.

- 19) RASMUSSEN DD, MITTON DR, LARSEN SA, YELLON SM. Aging-dependent changes in the effect of daily melatonin supplementation on rat metabolic and behavioral responses. *J Pineal Res* 2001; 31: 89-94.
- 20) PRUNET-MARCASSUS B, DESBAZEILLE M, BROS A, LOUCHE K, DELAGRANGE P, RENARD P, CASTEILLA L, PÉNICAUD L. Melatonin reduces body weight gain in Sprague Dawley rats with diet-induced obesity. *Endocrinology* 2003; 144: 5347-5352.
- 21) WOLDEN-HANSON T, MITTON DR, McCANTS RL, YELLON SM, WILKINSON CW, MATSUMOTO AM, RASMUSSEN DD. Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. *Endocrinology* 2000; 141: 487-497.
- 22) RASMUSSEN DD, BOLDT BM, WILKINSON CW, YELLON SM, MATSUMOTO AM. Daily melatonin administration at middle age suppresses male rat visceral fat, plasma leptin, and plasma insulin to youthful levels. *Endocrinology* 1999; 140: 1009-1012.
- 23) LIMA FB, MACHADO UF, BARTOL I, SERAPHIM PM, SUMIDA DH, MORAES SM, HELL NS, OKAMOTO MM, SAAD MJ, CARVALHO CR, CIPOLLA-NETO J. Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. *Am J Physiol* 1998; 275: 934-941.
- 24) PICINATO MC, HABER EP, CARPINELLI AR, CIPOLLA-NETO J. Daily rhythm of glucose-induced insulin secretion by isolated islets from intact and pinealectomized rat. *J Pineal Res* 2002; 33: 172-177.
- 25) EINHORN D, REAVEN GM, COBIN RH, FORD E, GANDA OP, HANDELSMAN Y, HELLMAN R, JELLINGER PS, KENDALL D, KRAUSS RM, NEUFELD ND, PETAK SM, RODBARD HW, SEIBEL JA, SMITH DA, WILSON PW. American College of Endocrinology position statement on the insulin resistance syndrome. *Endocr Pract* 2003; 9: 237-252.
- 26) LA FLEUR SE, KALSBECK A, WORTEL J, FEKKES ML, BUIJS RM. A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus. *Diabetes* 2001; 50: 1237-1243.
- 27) REITER RJ, TAN DX, KORKMAZ A. The circadian melatonin rhythm and its modulation: possible impact on hypertension. *J Hypertens* 2009; 27(Suppl 6): 17-20.
- 28) LA FLEUR SE, KALSBECK A, WORTEL J, VAN DER VLIET J, BUIJS RM. Role for the pineal and melatonin in glucose homeostasis: pinealectomy increases nighttime glucose concentrations. *J Neuroendocrinol* 2001; 13: 1025-1032.
- 29) OKAMOTO Y. Adiponectin provides cardiovascular protection in metabolic syndrome. *Cardiol Res Pract* 2011; 2011: 313179.