

Experimental models of melatonin-deficient hypertension

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1. ABSTRACT

Melatonin secreted by the pineal gland plays an important role in the regulation of blood pressure (BP) and its administration reduces hypertension both in animals and humans. There are two experimental models of melatonin-deficient hypertension: one induced by pinealectomy and another by continuous 24 hour exposure to light. Both models cause melatonin deficiency and prevent darkness-mediated nocturnal melatonin secretion and are associated with increased BP and myocardial, vascular and renal dysfunction. These models also lead to neurohumoral activation of the renin-angiotensin system, sympathetic nervous system, adrenocorticotrophin-glucocorticoid axis and cause insulin resistance. Together, these alterations contribute to rise in blood pressure by vasoconstrictive or circulatory fluid volume overload. The light induced hypertension model mimics the melatonin deficiency in patients with insufficient nocturnal BP decline, in those who have night shift or who are exposed to environmental light pollution. For this reason, this model is useful in development of anti-hypertensive drugs.

2. INTRODUCTION

Despite extensive basic research and unremitting clinical efforts devoted to hypertension treatment, the therapeutic achievements for elevated blood pressure are far from being sufficient. This may be due to the fact that essential hypertension is a condition of multifactorial origin and its pathogenesis is still not quite clear. Mechanisms of hypertension involve alterations in central regulatory pathways, vascular disturbances, and neurohumoral imbalance. Melatonin, the secretory product of pineal gland, based on recent findings, appears to be a significant factor in blood pressure regulation (1-5).

3. MELATONIN-BLOOD PRESSURE INTERACTION: RESEARCH APPROACH

There are two principal ways to show that a particular inherent substance contributes to a given physiological or pathological state, i.e., 1) inducing a condition that causes an excess of the agent in question or 2) inducing a deficiency of the agent relative to its physiological concentrations.

3.1. Treatment of hypertension with melatonin

Melatonin administration (5-10 mg/kg / day) reduced blood pressure slightly but reliably in rats with L-NAME-induced hypertension (6), in adult (7,8) or young (9,10) spontaneously hypertensive rats (SHR), in stress-induced hypertension (11,12) or in rats with metabolic syndrome (13). Melatonin (1-5 mg/day) reduced blood pressure in healthy men (14) and women (15) with normal blood pressure, in patients with metabolic syndrome (16), night-time blood pressure in otherwise untreated hypertensive men (17), non-dipping women (18), patients with nocturnal hypertension (19), adolescents suffering from type 1 diabetes mellitus (20), and in non-dippers with coronary artery disease (21). The meta-analysis of human studies of melatonin administration to hypertensive patients concluded that only treatment with long-acting, controlled-released melatonin reduces reliably nocturnal blood pressure (2). Melatonin administration, in animals, in addition to reducing high blood pressure, protected against hypertensive target organ damage at the level of the heart (2,4,8,23,24), vessels (25,26) and the kidney (7).

3.2. Pinealectomy and continuous light-induced hypertension

Pineal gland is the major source of circulating melatonin (27,28) and melatonin is the principal secretory product of the pineal gland (29,30); its rhythm modulates circadian rhythmicity of a number of physiological functions (28,31). The pineal gland receives a neural input from the suprachiasmatic nucleus, the central biological clock, that results in the circadian rhythm of melatonin production and release, ensuring physiological synchronization with the light-dark cycle. Melatonin blood levels are always low during the daytime and highest during the night (28,32).

There are two established experimental approaches that severely compromise melatonin production and disturb its regulatory role in daily rhythmicity, i.e. pinealectomy or continuous light exposure.

3.2.1. Pinealectomy-induced hypertension

The most commonly used method for the surgical removal of the pineal gland in rodents was described by Hoffman and Reiter in 1965 (33). This method was refined later with the use of magnification which facilitated the localization, visibility and removal of the pineal gland from its location between the hemispheres and the cerebellum with limited injury to the dura mater, the venous sinuses and the brain (34). The new procedure may also reduce the variability of experimental outcomes.

Pinealectomy was originally used to identify the relation between melatonin rhythm and the regulation of seasonal reproduction (35-37). That pinealectomy induces a rise in blood pressure was first reported by Zanoloni et al. (38) and the hypotensive effect of chronic melatonin treatment in pinealectomy-mediated hypertension was described in 1976 (39). The arterial blood pressure rise takes place within 15 days after the pinealectomy and persists for 30 and 60 days afterwards and the blood pressure spontaneously returns to normal range after three months (40). Pinealectomy evokes a slight blood pressure elevation

especially in the prepubertal (43-day-old) and possibly less so in postpubertal (55 day-old) male rats. Real hypertension (with blood pressure above 150 mmHg) is induced by 1% saline consumption by pinealectomized rats (41), since it unmasks hypertension in the "pre-hypertensive" pinealectomized animals.

Pinealectomized hypertensive rats show increases in heart weight and microscopically demonstrable myocardial fibrosis and myxomatous degeneration of the cardiac valves (30) along with the perivascular fibrosis of the renal tissue with the arteriolar thickening (40). Moreover, in pinealectomized animals food and water intake is greater resulting in an increase in body weight, but both return to normal two to three months after pinealectomy like the blood pressure (40).

The fact that pinealectomy induces only transitory hypertension is determined by temporary alterations in vascular reactivity. Although vascular responses to norepinephrine, serotonin and angiotensin II were greater than that of control rats one week after pinealectomy but the long-term (two months after pinealectomy) endothelium-intact and -denuded arteries showed almost identical contractions in response to phenylephrine, serotonin, angiotensin II, vasopressin and calcium (42), whereas only endothelin-induced contractions in the endothelium-denuded vessels isolated from the pinealectomized rats were increased (43). This modification in vascular reactivity may be partly induced by metabolic and biochemical changes seen in the pinealectomized animals. Zinc levels have been shown to increase in thoracic aorta, as were serum concentrations of cholesterol, sodium (44) and leptin (45). These changes may be involved in the alteration of vascular function through endothelium-dependent or -independent mechanisms.

3.2.2. Continuous light-induced hypertension

It is generally known that application of artificial light during the period of darkness reduces the production and secretion of melatonin from the pineal gland (46-48). Long-term exposure of rats to constant 24h/day light dissociated the circadian activity rhythms in female rats (49). Constant low intensity light (5-10 lux) essentially abolishes circadian rhythmicity in cardiovascular parameters including blood pressure and heart rate and blood pressure rhythm was not restored by intraperitoneal injection of 1mg/kg melatonin (50). Similarly, continuous light exposure of five week old rats for 17 weeks completely suppressed blood pressure and heart rate circadian rhythms as measured by telemetry. Subcutaneous melatonin injections at the theoretical onset of darkness for three weeks or the transfer of these rats to a standard 12:12-hour light/dark cycle failed to restore circadian rhythm of blood pressure. Since anatomical (by cervical ganglionectomy) or pharmacological (with 6-hydroxydopamine) sympathectomy also completely abolished the circadian rhythm of cardiovascular parameters, it was suggested that continuous light-induced cardiovascular rhythms imbalance is induced through the light-mediated increase of sympathetic outflow (51).

Continuous 24-hour light exposure of rats for six weeks induced hypertension (23), which was less pronounced

Table 1. Melatonin effects related to blood pressure regulation

Melatonin effects	Pathophysiology	References
Circadian rhythms control	Transducer of light/dark information	28, 32, 67, 60
Reduction of free radical burden	Scavenging of oxygen and nitroso radicals	1, 10, 6, 25, 48, 67, 71-73 75, 77, 78
	Stimulation of antioxidant enzyme expression	
Antiinflammation	Blockade of expression of proinflammatory cytokines	1, 32, 48, 70
Improving endothelial function	Increasing nitric oxide bioavailability	3, 25, 66, 74
Antiadrenergic and parasympathomimetic action	Reduction of norepinephrine level in serum	3,4, 15, 66, 83, 85
	Reduction of β_2 receptors in the heart	
	Improving baroreflex function	
Antithrombotic action	Reduction of platelet aggregation	1, 60, 66
Antilipidemic effect	Depression of serum LDL cholesterol level	1, 16, 66, 73
	Reduction of LDL cholesterol oxidation	
Improvement of glucose metabolism	Increased insulin sensitivity	89, 90
	Enhancement of GLUT4 expression	
Body weight control		27, 63, 78
Vasodilatation	Reduction of norepinephrine release	1, 3, 16, 66, 83, 84
	Induction of NO formation	
	Interaction with MT receptors	
Hypotensive effect in experimental hypertension	Complex mechanism	8-13, 26, 30, 38, 40, 41, 74
Hypotensive effect in human hypertension	Complex mechanism	15-22, 70, 77, 84
Prevention of remodeling of the left ventricle	Antifibrotic effect	4, 6, 8, 9, 23, 24,
	Antihypertrophic effect	
	Antischematic effect	
Prevention of remodeling of the aorta and small arteries	Antifibrotic effect	25, 26, 70, 01
	Antihypertrophic effect	
Kidney protection	Antifibrotic effect	7
	Anti-inflammatory effect	

than in L-NAME-treated rats (52-54) or in spontaneous hypertensive rats (SHR) (8). Moderate hypertensive response resulted in left ventricular hypertrophy (LVH) and fibrotic rebuilding of the LV characterized by an increased concentration of hydroxyproline in both soluble (non-mature collagen with little cross-linking) and insoluble (mature, with elevated cross-linking) collagen fractions and in total collagen (the sum of soluble and insoluble collagen) (23). In a continuous light exposure combined with L-NAME administration model, more pronounced hypertension and greater fibrosis development was observed. Interestingly, melatonin administration did not prevent LVH development, but partly limited fibrosis in continuous light plus L-NAME model of hypertension (23).

4. POTENTIAL PATHOGENESIS OF PINEALECTOMY- AND CONTINUOUS LIGHT-INDUCED HYPERTENSION

4.1. Melatonin deficiency

Pinelectomy and continuous light exposure reduce circulating melatonin levels. Experimental pinelectomy reduces both nocturnal and day time melatonin levels (39), however, it does not lead to a complete eradication of melatonin from plasma, since the pineal gland is the main but not an exclusive source of melatonin. Gastrointestinal tract produces significant amounts of melatonin (28,55,56) and this extra-pineal production may be even increased after pinelectomy in a compensatory manner; thus melatonin levels may be significantly reduced but not totally absent in the blood of pinelectomized animals. Indeed, a patient after pinelectomy showed no nocturnal increase in blood melatonin, but it was still detectable (57). Interestingly, pinelectomy increased melatonin content in all subcellular compartments in rats (58). The urinary excretion of 6-sulphatoxymelatonin (aMT6s), a metabolite of melatonin, was depressed in pinelectomized rats during both the day and night (59).

In a model of inappropriate nocturnal light exposure, melatonin levels are markedly inhibited during the normal darkness period. Hamsters maintained in either long (light-dark 16:8) or short (light-dark 8:16) photoperiod showed daily rhythms in both pineal melatonin concentration and in aMT6s excretion (60,61). However, the amplitude and the duration of nocturnal aMT6s excretion was lower when daily light exposure was longer (62). Constant 24-hour light-exposure of rats abolished the nocturnal rise in aMT6s (59) and the melatonin levels in constant light exposed animals were significantly lower than those kept in a 12/12 light: dark or in constant darkness (63). Melatonin levels in healthy human subjects under constant white light of 50 lux were suppressed by 53% compared to total darkness (64). Similarly, sea bass exposed to continuous light showed no elevation in plasma melatonin during the night hours (65) indicating that the compromised melatonin production by continuous light exposure is not restricted to mammals.

Pinelectomy and continuous light exposure cause a relative melatonin deficiency by eliminating the circadian production of melatonin, and the resulting hypertension is referred as „melatonin-deficient” hypertension.

4.2. Pathophysiology of the melatonin blood pressure reducing effect

Melatonin exerts a number of pleiotropic actions which might be protective within the cardiovascular system and involved in the prevention of hypertension and end organ-damage (Table 1); additionally, melatonin could cause a reversal of already established elevated blood pressure and its negative consequences (3,4,66,67). Melatonin has extraordinary antioxidant potential and reduces the level of oxidative stress within different organs and tissues (68-71). Melatonin and several of its metabolites have the ability to combat the damage inflicted by both oxygen- and nitrogen-

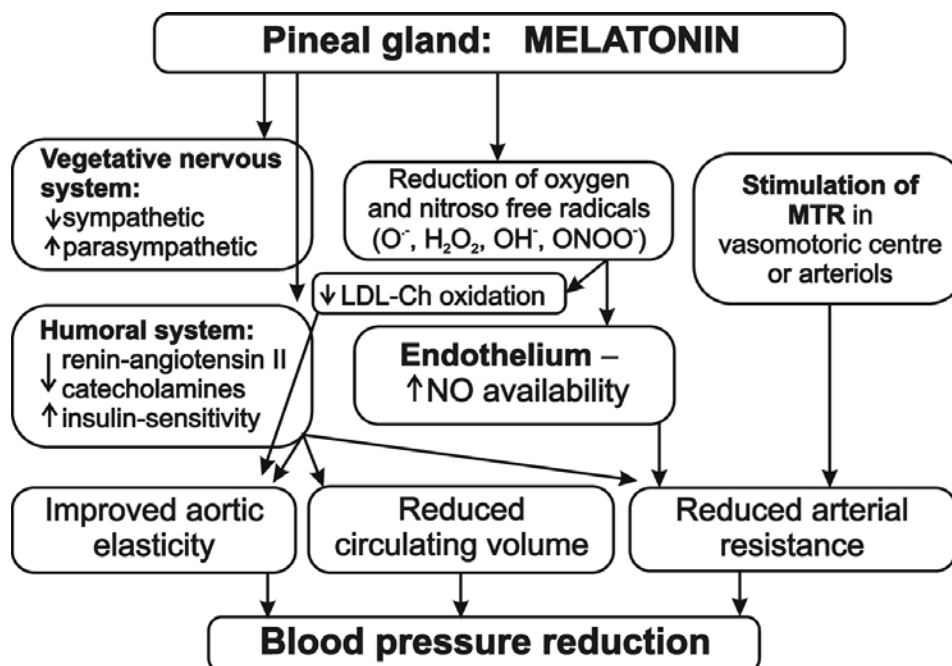


Figure 1. Blood pressure regulating factors and potential benefits of melatonin interactions. Melatonin induces the relative dominance of the parasympathetic nervous system, attenuates the neurohumoral activation (level of norepinephrine, renin and insulin) and reduces oxidative burden improving thus endothelial function and increasing nitric oxide bioavailability. Furthermore, it interferes with central vasomotor center through specific melatonin receptors (MTR). These effects, each alone or in synergy, may reduce blood pressure through the attenuation of peripheral arterial resistance, reduction of circulating fluid volume or improvement of the elasticity of large vessels.

derived free radicals (70,72,73) and its lipophilic action enables it to cross the cell membrane and extend its protective action to subcellular structures, such as mitochondria or nuclear DNA (2,58,74). Furthermore, melatonin stimulates the activity of enzymes that scavenges radicals (10,75-78). The metabolic derivatives of melatonin are equally as effective as melatonin in relieving the free radical burden (2,67).

It is noteworthy that in the combined model of continuous light plus L-NAME treatment, melatonin prevented an increase in the levels of malondialdehyde and advanced oxidative protein products in plasma (8). Melatonin increases the production of nitric oxide (NO) and reduces its degradation by free radicals or asymmetric dimethylarginine, thus enhancing NO bioavailability (25,74,79) and exerting cardiovascular protection through vasodilatation, hypotension, and inhibition of pathologic growth of the heart and vessels (80-82).

The interference of melatonin with the peripheral and central autonomic system favoring the parasympathetic over the sympathetic neural tone is of significant importance for cardiovascular system regulation (15,83). Besides its receptor-independent pleiotropic effects, melatonin may act on blood pressure through the specific melatonin binding sites, i.e. G-protein-coupled melatonin receptor subtypes localized in peripheral vessels and in blood pressure regulating structures of the central nervous system (Figure 1) (11,67,78,84).

4.3. Other pathogenic factors of melatonin-deficient hypertension

Melatonin has a number of physiological attributes, that may contribute to its ability to downregulate blood pressure. A reduction in melatonin levels may be the main or at least a triggering factor responsible for hypertension development in the two models of its relative deficiency described herein, i.e. pinealectomy and light induced hypertension. However, alterations in other factors, particularly the sympathetic nervous system and the renin-angiotensin system may contribute to hypertension development.

Myocardial fibrosis observed as a consequence of continuous light exposure (23) or pinealectomy (30) may be associated with pro-proliferative effects of angiotensin II or aldosterone. Indeed, angiotensin converting enzyme (ACE) expression in the LV increased in continuous light- and continuous light plus L-NAME-induced hypertension and was prevented by melatonin and ACE-inhibitor captopril; moreover, both compounds attenuated hypertension and LV remodeling (23). Plasma renin activity was significantly increased five weeks after pinealectomy, which likely reflected increased sympathetic activity as the most probable cause of hypertension in pinealectomized animals (85). Although plasma concentration of dopamine and norepinephrine were reduced in female rats exposed to constant light for six weeks, epinephrine synthesis in the adrenal gland was increased (86). The rise in excitability and

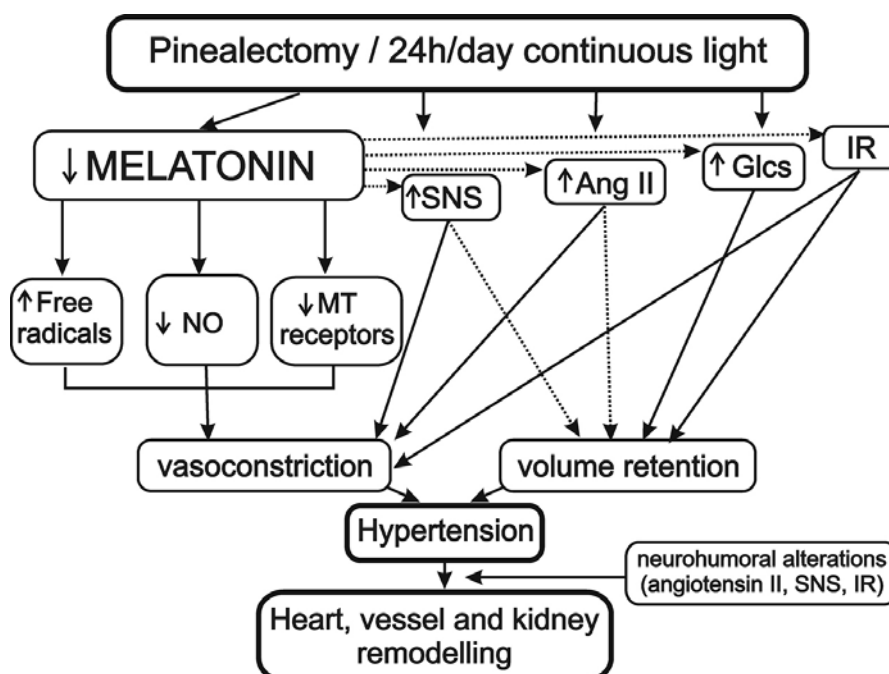


Figure 2. Etiopathogenesis of melatonin-deficient hypertension and end organ damage. Experimental pinealectomy or exposure of animals to chronic continuous illumination results in reduced melatonin levels. Melatonin deficiency may lead to hypertension development through the shortage of melatonin receptor dependent or independent (oxidative stress, deficit of nitric oxide) protective mechanisms. Also a melatonin deficiency, and alteration of several neurohumoral systems (sympathetic nervous system, renin-angiotensin system, adrenocorticotropin-glucocorticoid axis, insulin resistance) may enhance peripheral vasoconstriction or blood volume, both of which would increase blood pressure. Hemodynamic stress occurring along with neurohumoral alterations induces remodeling of the heart, vessels and kidney. (NO-nitric oxide, MT receptors – specific melatonin MT1 or MT2 receptors, SNS-sympathetic nervous system, Ang II - angiotensin II, Glc – glucocorticoids, IR -insulin resistance)

irritability observed in rats exposed to continuous light (63) also suggests augmented sympathoexcitation.

Besides the potential role of catecholamines and angiotensin II, adrenocorticotrophic hormone (ACTH)-glucocorticoid axis activation should be considered as a factor in melatonin deficiency hypertension. The volume of zona fasciculata of the adrenal gland and the size of individual cells and their nuclei in this layer were enhanced while serum corticosterone concentrations were elevated after 95 days of exposure to continuous light of 600 lux (87); this was obviously the result of increased ACTH production observed at both six weeks (86) or after 95 days of chronic light exposure (88). Corticosteroids, due to their sodium and water sparing effects, increase the circulatory fluid volume and thereby could contribute to hypertension development.

Serious alterations in metabolism are observed in both pinealectomized and in constant illumination exposed animals. Continuous light exposure of rats decreases locomotor activity, increases feed intake, and thus visceral obesity (63). Pinealectomy reduces adipose tissue responsiveness to insulin (89), induces insulin resistance (90) and glucose intolerance (89). Furthermore, enhanced leptin production (89), increased cholesterol concentrations (44), structural deterioration of the renal tissue (40) and higher serum sodium, creatinin and urea levels (44) were observed in pinealectomized rats. These alterations suggest that in

melatonin-deficiency metabolic syndrome is prone to develop (27), and this may contribute to the hypertension development via variable vasoconstrictive and circulating volume enhancing mechanisms.

Finally, pinealectomy compromises the cerebral vasculature by inducing atrophy and reducing the distensibility of cerebral arterioles (91) making cerebral tissue more sensitive to ischemia (92) and this might compromise the blood pressure regulatory role of the central nervous system.

Based on the above description it is not unreasonable to suggest that melatonin deficiency in pinealectomized or continuous light exposed animals triggers a sequence of neurohumoral and metabolic imbalances, which may contribute to the development of hypertension and target organ damage (Figure 2).

5. SUMMARY AND PERSPECTIVES

Since hypertension is a multifactorial disease, new innovative experimental approaches for testing the protective potential of novel molecules are needed. Although, pinealectomy or continuous illumination-induced hypertension has been known for many years, their wide use as models of hypertension has been unjustifiably neglected. The 24h/day light-induced inhibition of the nightly increase

in melatonin secretion is noninvasive and a more physiological approach to reduce melatonin levels than pinealectomy, which reduces both day and night melatonin secretion (51,59,93). Inhibition of circadian rise in melatonin by continuous light mimics its profile in patients with an insufficient decline in blood pressure (non-dipping) (70); the week increase in urinary aMT6s reflects a blunted elevation in serum melatonin during the night in these subjects (94). An insufficient nocturnal decline in blood pressure puts greater burden on the cardiovascular system and increases morbidity and mortality independent of other risk factors (95). It is therefore reasonable to suppose that illumination during the period of natural darkness, a general phenomenon due to world-wide urbanization, diminishes nocturnal melatonin secretion. Disturbed photoperiodic environment in the form of light exposure at night also causes disturbances in the function of the suprachiasmatic nucleus (central rhythm generator), which then transfers this inappropriate and confusing information to both the pineal gland and the cells in all peripheral tissues. This results in insufficient decline in night-time blood pressure or even in blood pressure rise, but also contributes to metabolic disorders, sleep disturbances and oncological pathologies (96-98).

The continuous light-induced hypertension is a complex phenomenon involving, in addition to a deficiency of melatonin, also several other metabolic and neurohumoral disturbances including insulin resistance, activation of renin-angiotensin and sympathetic nervous system perturbation, all of which may contribute to the pathogenesis of essential hypertension in humans. Moreover, the generalized rise in blood pressure in older people may also be related to a relative melatonin deficiency, since this essential molecule declines as humans age (70). The model of constant illumination-induced hypertension may therefore be expected to attract growing interest of experimental cardiologists in the near future.

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Abbreviations: BP: blood pressure, L-NAME: NG - nitro-L-arginine methyl ester, SHR: spontaneously hypertensive rat, aMT6s: 6-sulphatoxymelatonin, ACTH: adrenocorticotrophic hormone, NO: nitric oxide, MT receptors: specific melatonin MT1 or MT2 receptors, SNS: sympathetic nervous system, Ang II: angiotensin II, Glc: glucocorticoids, IR: insulin resistance

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