Influences of melatonin administration on the circulation of women

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Cagnacci, Angelo, Serenella Arangino, Marco Angiolucci, Elisabetta Maschio, and Gian Benedetto Melis. Influences of melatonin administration on the circulation of women. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R335–R338, 1998.—The cardiovascular effects induced by the daytime administration of melatonin (1 mg) were compared with those of placebo in 17 young, healthy, early follicular-phase women. Compared with placebo, the administration of melatonin modified, within 90 min, the pulsatility index (PI), evaluated by color Doppler ultrasound, of the internal carotid artery, abdominal aorta, and axillary artery. The effect was linearly related to baseline PI, higher baseline PI being associated with greater PI declines. Melatonin administration significantly decreased mean PI of internal carotid artery ($P < 0.02$), systolic and diastolic blood pressure ($P < 0.01$), and norepinephrine levels evaluated after 5 min of standing position ($P < 0.02$). Heart rate and supine catecholamine levels were not modified. These data indicate that in young, healthy women the administration of 1 mg of melatonin greatly influences artery blood flow, decreases blood pressure, and blunts noradrenergic activation. Clinical implications of present data are worthy to be fully explored.

blood flow; catecholamine; blood pressure; arteries

MELATONIN SECRETED by the pineal gland in a suprachiasmatic nuclei-driven circadian fashion is involved in the regulation of the circadian rhythm of several biological functions (3, 17). Experimental data have also suggested a possible influence of melatonin on circulatory functions of animals. The administration of melatonin reduces blood pressure in normal (7), pinealectomized (10), and spontaneously hypertensive (12) rats, whereas hypertension is induced by pinealectomy in rats (11, 29). Melatonin influences the turnover of catecholamine in perivascular nerves (26), influences pulmonary artery and vein via endothelium-dependent factors (27), induces relaxation of rat aorta (25), directly influences Ca^{2+}-dependent cardiac sarcolemma adenosinetriphosphatase (6), and modifies catecholamine metabolism in the heart (23). Receptors for melatonin have been detected in the walls of cerebral and caudal arteries of rats (5, 24), and melatonin both decreases blood flow to the animal brain (4) and enhances the constriction of rat caudal artery induced by norepinephrine (24). These melatonin-induced circulatory modifications have also been considered relevant for the control of rat body temperature (24). Receptors for melatonin have been detected in the wall of cerebral arteries of subhuman primates (22), and clear influences of melatonin in the control of body temperature have been reported in humans (3). Despite these effects, whether melatonin is capable in humans of influencing circulatory functions has never been investigated. Accordingly, in the present study the cardiovascular modifications induced by the administration of melatonin were studied in young, healthy women.

MATERIALS AND METHODS

The study was performed in 17 young, normal weight, healthy women (aged 23–29 yr), within 15% of their ideal body weight. Women on no medication were studied in the early follicular phase of the menstrual cycle (*days 4-6*) to eliminate cardiovascular modifications related to fluctuating endogenous gonadal hormones (9). All women were entrained to a normal day-night rhythm with a regular sleep pattern. Furthermore, each woman was requested to keep a regular sleep schedule (from 1030–1130 to 0700–0800).

Between 1400 and 1800 of 2 consecutive days, each woman received 90 min before investigation, randomly and in a double blind fashion, a capsule containing either placebo or melatonin (1 mg). The first day melatonin or placebo was randomly assigned, and on the second day the alternate pill was assigned. At the same time, a two-way stopcock heparinized polyethylene catheter was inserted in an antecubital vein for blood drawing. Each woman was requested to relax supine in a low-noise, low-light, constant-temperature (20–21°C) environment for the 30 min preceding the investigation.

Blood flow velocity waveforms were evaluated by color Doppler ultrasound (Acuson 128XP/10OB, Acuson, Mountain View, CA) after the procedure previously described by Gangar et al. (9). The investigation was initiated when heart rate and systolic and diastolic blood pressure, recorded with an automatic device (Dinamap 845 XT, Critikon, Tampa, FL) at 5-min intervals, respectively, varied by <5 beats/min and by <5 mmHg over two consecutive readings. Immediate preinvestigation heart rate and blood pressure values were recorded for statistical analysis. The pulsatility index (PI), which is the ratio of maximal Doppler shift in systole on the mean Doppler shift of the entire waveform, is thought to directly represent downstream vasomotor state (9), and it was measured three times for each evaluation from the blood flow velocity waveform. The mean of the three measures was used for the statistical analysis. The internal carotid artery and the axillary artery were sampled with a 7.0-MHz linear transducer. The former was sampled 1.5 cm above the common carotid artery, and the latter at its exit from the axilla. The abdominal aorta was sampled with a 3.5-MHz linear transducer, 1.5 cm above its bifurcation. Doppler evaluations were performed by the same operator. The intraoperator coefficient of variation was <3.5%.

At the end of the Doppler investigation, blood samples for catecholamine and melatonin determination were collected, with women still in supine position. Another blood sample was collected after 5 min of upright position for catecholamine determination.

For the analysis of melatonin, the blood was collected in glass tubes and, after centrifugation, the serum was stored at −20°C until assayed in duplicate by radioimmunoassay. The

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antiserum to melatonin raised in rabbits (Stockgrand LTD, Guildford Surrey, UK) was used at a dilution of 1:75, with ^{125}I-labeled melatonin (Amersham Italia, Milan, Italy) as tracer. Melatonin was purchased from Sigma (St. Louis, MO). A solid-phase separation system (Dabsep; Stockgrand LTD) was used to separate bound from unbound melatonin. The assay has a sensitivity of 1.5 pg/ml (6.5 pmol/l), and intra- and interassay coefficients of variations are <5 and 6.3%, respectively.

For the analysis of catecholamine, the blood, collected on ice in a glass tube containing EDTA, was immediately centrifuged and stored for a maximum of 3 days at −25°C before being assayed for norepinephrine and epinephrine levels. After extraction with alumina, the analysis of catecholamine was performed in duplicate, by ion pair high-performance liquid chromatography using a Supelcosil LC-18-DB column (Supelchem, Milan) and an electrochemical detector (M-460, Water), as previously described (19). The mobile phase was a sodium acetate buffer (160 mmol/l; pH 4.05), with sodium octyl sulfate (0.16 mmol/l), Na_2EDTA (1 mmol/l), and acetonitrile (15 ml/l) at a flow rate of 1.0 ml/min. Samples of each subject were analyzed in the same assay to eliminate interassay variability. For each sample, peak height ratios to internal standard were calculated and introduced into the calibration curve equation obtained by linear regression analysis. Spiked biological samples gave a linear response in the range of interest for each compound. The intra-assay coefficient of variation of the method was <5.5%.

Statistical analysis of the results was performed by the *t*-test for paired data. PI changes induced by melatonin were regressed on baseline PI observed on the placebo day by linear regression analysis.

RESULTS

No signs of atheromatous plaques were detected in any vessels investigated.

Melatonin levels were 15.1 ± 5.1 pmol/l on the placebo day and 8,540 ± 2,650 pmol/l on the melatonin day.

Compared with placebo, the administration of melatonin induced a significant decrease in internal carotid artery PI (Table 1). The net PI change (−0.53 ± 0.14, $P < 0.02$) was directly correlated with the baseline PI (placebo day) ($y = -0.763x + 0.6$; $r = 0.787$; $P < 0.0003$) (Fig. 1) and was more pronounced (up to −68%) when PI of the placebo day was greater. Such as on the internal carotid artery, melatonin exerted an effect on PI of axillary artery and abdominal aorta that was dependent on the previous status. Low baseline PI was increased, whereas high baseline PI was decreased by the administration of melatonin, following a simple linear regression (axillary artery: $y = -0.785x +$

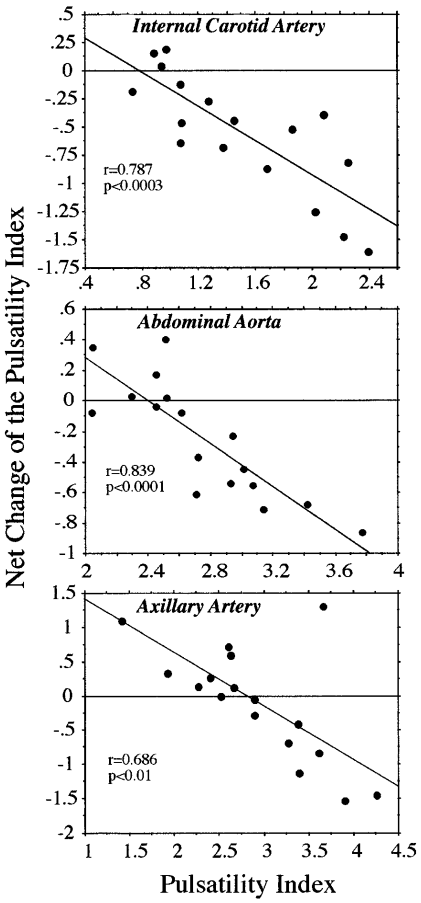

Fig. 1. Correlation between changes in pulsatility index (PI) (difference between melatonin and placebo day) and baseline PI (placebo day) of internal carotid artery, abdominal aorta, and axillary artery of 17 healthy young women.

Table 1. *Mean values and differences between melatonin and placebo day of V_{max} and of PI evaluated in internal carotid artery, abdominal aorta, and axillary artery of 17 young healthy women*

	Placebo	Melatonin	Net	*P*
Internal carotid artery				
V_{max}	0.484 ± 0.04	0.448 ± 0.028	−0.007 ± 0.05	NS
PI	1.482 ± 0.14	0.952 ± 0.09	−0.531 ± 0.14	0.002
Abdominal aorta				
V_{max}	0.548 ± 0.04	0.493 ± 0.02	−0.059 ± 0.04	NS
PI	2.781 ± 0.11	2.515 ± 0.07	−0.26 ± 0.10	NS
Axillary artery				
V_{max}	0.574 ± 0.06	0.501 ± 0.04	−0.07 ± 0.07	NS
PI	2.96 ± 0.18	2.84 ± 0.16	−0.02 ± 0.02	NS

Values are means ± SE [maximal Doppler shift in systole (V_{max}) in m/s]. Net, mean values and differences between melatonin and placebo day; PI, pulsatility index.

Table 2. *BP_{sys}, BP_{dia}, BP_{me}, and HR measured in supine position and circulating levels of NE and Epi measured in supine position and in orthostatism after the oral administration of placebo or melatonin (1 mg)*

	Placebo	Melatonin	*P*
BP_{sys}, mmHg	110.8 ± 2.9	101.1 ± 1.6	<0.01
BP_{dia}, mmHg	67.0 ± 2.9	59.3 ± 2.8	<0.01
BP_{me}, mmHg	81.3 ± 3.1	72.2 ± 3.1	<0.01
HR, beats/min	74.5 ± 1.9	73.9 ± 2.7	ns
NE_{clino}, pg/ml	258.5 ± 31.2	239.6 ± 35.6	ns
NE_{ortho}, pg/ml	448.7 ± 48.2	341.3 ± 32.5	$P < 0.02$
Epi_{clino}, pg/ml	23.3 ± 2.9	22.9 ± 2.0	ns
Epi_{ortho}, pg/ml	23.9 ± 4.6	25.1 ± 4.8	ns

Values are means ± SE. BP_{sys}, BP_{dia}, BP_{me}, systolic, diastolic, and mean blood pressure, respectively; HR, heart rate; NE, norepinephrine; Epi, epinephrine; Epi_{clino}, NE_{clino}, Epi_{ortho}, NE_{ortho}, Epi or NE measured in supine position and orthostatism, respectively. $BP_{me} = BP_{sys} - BP_{dia}/3 + BP_{dia}$.

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2.20, $r = 0.686$, $P < 0.01$; abdominal aorta: $y = -0.705x + 1.697$, $r = 0.839$, $P < 0.0001$) (Fig. 1).

In conjunction with modifications of artery PI, melatonin reduced systolic (-9.5 ± 2.8 mmHg, $P < 0.01$), diastolic (-7.5 ± 1.4 mmHg, $P < 0.01$), and mean blood pressure (-7.4 ± 1.5 mmHg, $P < 0.01$), all evaluated after 30 min of supine position in a relaxed environment (Table 2). Heart rate was not modified. Epinephrine and norepinephrine levels evaluated in supine position were not modified, whereas norepinephrine levels evaluated after 5 min of standing position were significantly reduced by melatonin (-105.1 ± 43.5 pg/ml, $P < 0.02$) (Table 2). Standing epinephrine levels were not modified (Table 2).

DISCUSSION

With the use of a noninvasive analysis of blood flow it has been possible to show an effect of melatonin on the circulation of healthy women. Although PI does not necessarily reflect regional blood flow, it is believed to reflect downstream vasomotor state and resistance to blood flow (9), and it is the only calculation that, in the absence of blood flow at the end of the diastole, can be performed to relate Doppler-derived parameters of blood flow in diastole to those of systole. PI was acutely modified, within 90 min, by the administration of melatonin.

The response to melatonin was not unidirectional and was dependent on the previous status. High PI values were reduced by the administration of melatonin, whereas low PI values were slightly increased. These modifications, evident for Doppler-derived parameters of blood flow of all investigated vessels, induced a significant decrease of mean PI only in the internal carotid artery. Either vessels downstream to the internal carotid artery were more responsive to the vasodilating effect of the hormone or their initial resistance was shifted toward higher values.

The interplay of several potential actions may explain the complex response of arteries to melatonin. Melatonin may bind to vessels via specific receptors (5, 22, 24) and interfere with artery response to catecholamine (24). Furthermore, melatonin may either reduce norepinephrine efflux from perivascular nerves (26) or reduce noradrenergic activity in both animals (7) and humans, as herein reported. All these modifications may play a role in mediating the mainly vasorelaxing activities of melatonin. On the other hand, melatonin has been reported to reduce prostaglandin production from rat hypothalamus (8) and nitric oxide production from rat cerebellum cells in vitro (16). Whether similar effects are exerted on the endothelium, melatonin may cause vasoconstriction through a reduction of vasodilating prostaglandins or nitric oxide (28). Indeed, a modulation of endothelium-dependent artery vasodilatation has been reported for melatonin in vitro (25).

A 5- to 10-mmHg decline in blood pressure is of relevance, because in hypertensive subjects a similar decrease in diastolic blood pressure is associated with a 20% reduction in cardiovascular mortality (18). The experimental design of the present study does not allow us to address whether lower or higher melatonin doses may induce different circulatory responses. The dose of melatonin administered was similar to that previously used to investigate influences of the hormone on human thermoregulatory functions (3) and in the range of those commonly used to treat desynchronized conditions or sleep disturbances (3). The levels reached by melatonin in blood were four to eight times greater than nocturnal peak values, and it is not known whether the observed results are physiologically meaningful. However, the effect of melatonin on some biological functions, such as the regulation of body temperature, seems to be a threshold phenomenon, physiological levels being as effective as those in the pharmacological range (3). In this case, present results may suggest that in humans blunted cardiocirculatory functions at night (15, 21) are, at least in part, due to the nocturnal secretion of melatonin.

Perspectives

Several clinical implications can be envisioned from the present data. The nocturnal production of melatonin may help protect against cardiocirculatory accidents, which occur more frequently in the periods of melatonin withdrawal, such as during morning hours, or in the periods of absent melatonin secretion, such as during daytime (13). Reduced levels of melatonin have been found in aged humans (14) and in subjects suffering from coronary artery diseases (2), and this reduction may contribute to a greater manifestation of cardiovascular pathologies. In addition, although levels of melatonin have not been measured in hypertensive subjects, the 7-day administration of the hormone at the dose of 2 mg/day as nasal spray has been reported to reduce blood pressure by 30 mmHg in subjects with essential hypertension (1). Thus melatonin administration seems to induce beneficial effects on circulation of humans, and noteworthy benefits may be obtained by the chronic supplement of melatonin, particularly in those subjects with cardiovascular risk factors. Extensive studies are needed to test this possibility.

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