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No Influence of Melatonin on Cerebral Blood Flow in Humans

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Melatonin has been attributed a role in a number of physiological processes. Changes in distal skin temperature and blood pressure after intake of melatonin suggest that melatonin induces peripheral vasodilation. The effect on the cerebral blood flow is still unknown. We examined the effect of a single pulse of melatonin on cerebral and peripheral blood flow, using the latter as a positive control. Ten male healthy volunteers (mean age, 22 ± 3.2 yr) participated in a double-blind, randomized, placebo-controlled, cross-over study. On one occasion 10 μg melatonin were infused iv, and on the other occasion saline was infused as the matching placebo. Cerebral blood flow was measured using phase contrast magnetic resonance imaging. Peripheral blood flow was determined from changes in the distal to proximal skin temperature gradient and finger pulse volume. Serum melatonin concentration increased from 12 ± 5 pg/ml at baseline to 487 ± 377 pg/ml at 5 min and 156 ± 68 pg/ml at 10 min after melatonin administration. There was no significantly different time course for cerebral blood flow and cerebrovascular resistance. Compared with placebo, melatonin significantly increased peripheral blood flow, as measured by distal to proximal skin temperature gradient and finger pulse volume. These data demonstrate that melatonin does not have an acute regulatory effect on cerebral blood flow in humans. (*J Clin Endocrinol Metab* 88: 5989–5994, 2003)

THE NOCTURNAL PINEAL hormone melatonin is involved in a number of physiological functions. In humans, melatonin favors the propensity to sleep (1), contributes to the nocturnal drop in core body temperature, and plays an important role in the circadian organization of biological rhythms (2, 3). Oral administration of pharmacological levels of melatonin gives a small decrease in blood pressure (4) and induces heat loss (5, 6), strongly suggesting that endogenous melatonin mediates peripheral vasodilation. The direct effect of melatonin on human cerebral blood flow (CBF) is still unknown. Melatonin receptors have been identified in human cerebral arteries (7). The finding that CBF is reduced at night (8), when melatonin levels are increased, suggest that endogenous melatonin might be involved in cerebral vasodilation. Pharmacological plasma levels of melatonin have been shown to induce a decrease in the pulsatility index of the internal carotid artery, suggesting cerebral vasodilation (9–11). The echo-Doppler-based pulsatility index is thought to reflect the downstream vasomotor state. It is, however, an indirect measure of CBF, which is also subject to operator variability. A better technique for CBF measurement is phase contrast magnetic resonance imaging (MRI), which we have recently shown to measure CBF quantitatively and accurately (12).

To investigate the acute effect of an iv bolus of melatonin on CBF, we performed a randomized, placebo-controlled, double-blind, cross-over study. We assessed CBF and peripheral blood flow (PBF) simultaneously, using the PBF as a positive control. We aimed to replicate a short-lived single pulse of melatonin with plasma concentrations in the low pharmacological range.

Abbreviations: CBF, Cerebral blood flow; DPG, distal to proximal skin temperature gradient; ECG, electrocardiogram; MAP, mean arterial pressure; MRI, magnetic resonance imaging; PAT, peripheral arterial tone; PBF, peripheral blood flow.

Subjects and Methods

Subjects

Ten young male healthy volunteers (mean age, 22 ± 3.2 yr; length, 1.84 ± 0.06 m; weight, 74.3 ± 7.1 kg) participated in the study. Physical and routine blood examination, electrocardiogram (ECG), and conventional MRI of the brain (transverse relaxation time-weighted fast spin echo and fluid attenuated inversion recovery) revealed no abnormalities. Exclusion criteria were self-reported sleep disorders, shift work or time-zone crossing travel within 1 month, current smoking, use of drugs or more than three alcoholic drinks a day, body mass index higher than 26 kg/m^2, hypertension, claustrophobia, dyslipidemia, diabetes mellitus, signs or symptoms of cardiovascular disease, or any other significant abnormality in physical examination, blood analysis, ECG, or standard MRI scan. Before the start of the experiment, subjects refrained from nonsteroidal antiinflammatory drug use for at least 10 d and from alcoholic and caffeine-containing beverages for at least 12 h. Subjects were requested to keep a fixed sleep schedule (2300–0700 h). The protocol was approved by the medical ethical committee of the Leiden University Medical Center and conformed to the principles outlined in the Declaration of Helsinki. All subjects gave written informed consent.

Procedures

During the experiments, subjects were kept in a supine position with their heads comfortably stabilized in a magnetic resonance system operating at a field strength of 1.5T (ACS-NT15, Philips Medical Systems, Best, The Netherlands) under continuous audio and video surveillance. There was continuous communication with the subjects to prevent fall-

TABLE 1. Serum concentrations of melatonin, epinephrine, and norepinephrine before and 5 and 10 min after melatonin or placebo administration

	Before administration	5 min after administration	10 min after administration
Melatonin (pg/ml)			
Melatonin occasion	12 ± 5	487 ± 377	156 ± 68
Placebo occasion	9 ± 3	9 ± 3	9 ± 3
Epinephrine (nmol/liter)			
Melatonin occasion	0.13 ± 0.07	0.14 ± 0.08	0.15 ± 0.08
Placebo occasion	0.11 ± 0.07	0.10 ± 0.06	0.10 ± 0.06
Norepinephrine (nmol/liter)			
Melatonin occasion	1.2 ± 0.3	1.2 ± 0.3	1.1 ± 0.4
Placebo occasion	1.4 ± 1.0	1.3 ± 0.9	1.3 ± 0.7

All values are the mean ± SD.

ing asleep. A deep antecubital vein was cannulated for blood sampling and infusion of melatonin or placebo.

A gradient echo, phase contrast MRI technique (transverse relaxation time/echo, 16/9 ms; flip angle, 7.5°; slice thickness, 5 mm; rectangle field of view, 75%; scan percentage, 80%; matrix, 256) with a velocity encoding of 100 cm/sec was used for nontriggered flow measurements in the basilar artery and both internal carotid arteries. Flow measurements were analyzed on a Sun UltraSparc 10 workstation with the internally developed software package FLOW (13). Total CBF was defined as the summed flow measured in the basilar artery and both internal carotid arteries (expressed as milliliters per minute). The method has been demonstrated to be sensitive to detect 15% changes in CBF as induced by hypoxia (14).

Although the most refined methods to obtain skin PBF are not compatible with the MRI environment, *i.e.* strain gauge venous occlusion plethysmography, we applied two noninvasive methods. First, PBF was estimated by the distal to proximal temperature gradient (DPG), which shows an outstanding correlation (r = 0.98) with more invasive methods (15). The DPG was derived from eight thermistors (P-8432, ITC, Tokyo, Japan) placed at the radial forearm, the palmar side of the fingertip, the shin, and the plantar side of the toe on both sides and was calculated by subtracting the four proximal from the four distal temperature assessments. Second, PBF was simultaneously assessed by means of peripheral arterial tone (PAT) photoplethysmography (Itamar Medical, Caesarea, Israel) (16). All signals were recorded on an Embla digital recorder (Flaga hf, Reykjavic, Iceland).

Heart rate was continuously monitored from a one-lead ECG and was read out in 5-min intervals. Blood pressure was measured semicontinuously with intervals of 3 min by use of an automatic device and was read out in 5-min intervals. All blood samples were collected in heparinized Vacutainer tubes (BD Biosciences, Franklin Lakes, NJ). After centrifugation, all plasma samples were immediately frozen at −20 C until analysis. Plasma melatonin concentrations were determined in duplicate by RIA with melatonin (Sigma-Aldrich Corp., St. Louis, MO), the antibody against melatonin (Stockgrand Ltd., Guildford, UK), and the radioactive tracer ^{125}I (Amersham Pharmacia Biotech, Little Chalfont, UK). Catecholamine concentrations were determined to exclude a possible confounding influence of changed noradrenergic activity on blood flow.

Study protocol

For each subject the study was performed on 2 separate study d with an interval of 1 wk. On each study day an iv bolus of either placebo or 10 μg melatonin was given. The sequence of bolus injections was randomized and given in a double-blind fashion. The study was performed between 1000–1800 h because endogenous melatonin production is suppressed during this time of day. After positioning in the scanner, an equilibrium period of 20 min allowed heart rate and blood pressure to stabilize. After stabilization of heart rate and blood pressure, baseline values for CBF, PBF, mean arterial pressure (MAP), and heart rate were measured, and blood samples for determination of various baseline plasma concentrations were taken. Subsequently, an iv bolus of either 10 μg melatonin or placebo (0.9% NaCl) was administered. After −5, 0, 5, 10, 15, 20, 25, and 30 min CBF, heart rate and MAP were determined, whereas DPG and PAT were assessed continuously. Blood samples for determination of plasma concentrations of melatonin and catecholamines were taken directly before and after 5 and 10 min of administration of the bolus injection.

Analysis

Cerebral vascular resistance was calculated by dividing MAP (milligrams of mercury) by total CBF (milliliters per liter). For the continuous DPG and PAT measurements, 5-min interval medians were calculated for the 5 min preceding and the 30 min following bolus injection, resulting in seven interval medians. In contrast, for CBF, cerebral vascular resistance, MAP, and heart rate, eight momentary values (−5 to 30 min) were used for analysis. After confirmation of nonsignificant differences on the preassessments using paired *t* tests, we used Δ values, *i.e.* the difference from the 5 min preinfusion measurement, for all calculations, figures, and statistical tests. PAT finger pulse volume signals were expressed as a percentage relative to the preassessment (16). Univariate linear mixed model analyses were used for all outcome variables, with the interaction between time and treatment condition as the main factor of interest. $P < 0.05$ was regarded as significant.

Results

None of the subjects fell asleep during the experiments. Six subjects reported that they had experienced a transient feeling of sleepiness on 1 d of the study, which turned out to be the melatonin treatment day for all reports. No other noticeable effects after the administration of either melatonin or placebo were observed.

The serum melatonin concentration increased from 12 ± 5 (mean ± SD) pg/ml at baseline to 487 ± 377 pg/ml at 5 min and 156 ± 68 pg/ml at 10 min on the days of melatonin administration (Table 1). The serum melatonin concentration was 9 ± 3 pg/ml at all three time points on the days of placebo administration. There were no differences between the catecholamine concentrations before and after the administration of either melatonin or placebo. None of the preinfusion outcome variables differed significantly between the melatonin and placebo days (Table 2; all $P > 0.25$).

There was no significantly different time course after the administration of melatonin and placebo for either CBF ($P = 0.75$) or cerebrovascular resistance ($P = 0.35$; Fig. 1). There was also no significantly different time course after the administration of melatonin and placebo for MAP ($P = 0.84$) or heart rate ($P = 0.95$; Fig. 2). PBF showed a markedly different time course after melatonin and placebo administration (Fig. 3). Compared with placebo infusion, melatonin significantly increased PBF as measured by DPG ($P < 0.001$) and PAT ($P = 0.003$). The maximal difference in PAT was 24% during the

20- to 25-min period after infusion. The maximal difference in DPG was 1.03 C at 15–20 min after infusion.

Discussion

The main finding of this study is that an experimentally induced increase in plasma melatonin concentration did not affect CBF and cerebrovascular resistance. The increase in PBF was, considering the literature, expected and served as a positive control, showing that melatonin levels were increased effectively.

The lack of effect on CBF cannot be attributed to insufficiently increased cerebral melatonin levels, because melatonin is a very lipophylic compound that readily crosses the blood-brain barrier even at low levels (17). Moreover, we had a statistical power of 80% for detecting a 13% difference in the time course of the CBF. For example, CBF changes of 15% have been reported during hypoxia (18) and changes of even 26% have been found during slow wave sleep (8). Hence, we conclude that melatonin does not acutely affect daytime CBF in healthy young males.

To mimic a single pulse of melatonin, a bolus of 10 μg melatonin was administered. The plasma levels of melatonin induced were relatively high compared with described endogenous levels (19). The plasma levels in our study, although pharmacological, were significantly lower than the levels reached in other studies using pharmacological doses of melatonin. Even 1 mg or oral melatonin induced plasma levels that were both higher (6360 pmol/liter corresponding to ±1500 pg/ml) and of a longer du-

TABLE 2. Values of all outcome parameters before melatonin and before placebo administration

	Preplacebo	Premelatonin
CBF (ml/min)	783 ± 151	840 ± 114
MAP (mm Hg)	77 ± 5.6	77.7 ± 5.8
CVR (mm Hg/min·ml)	0.10 ± 0.02	0.096 ± 0.02
DPG (C)	−4.6 ± 1.8	−4.8 ± 1.8
HR (beats/min)	65 ± 6.9	67 ± 7.7

All values are the mean ± SD. CVR, Cardiovascular resistance; HR, heart rate. Finger pulse volume as assessed by the PAT sensor, is omitted because its absolute value depends on placement on the fingertip and therefore has no meaning.

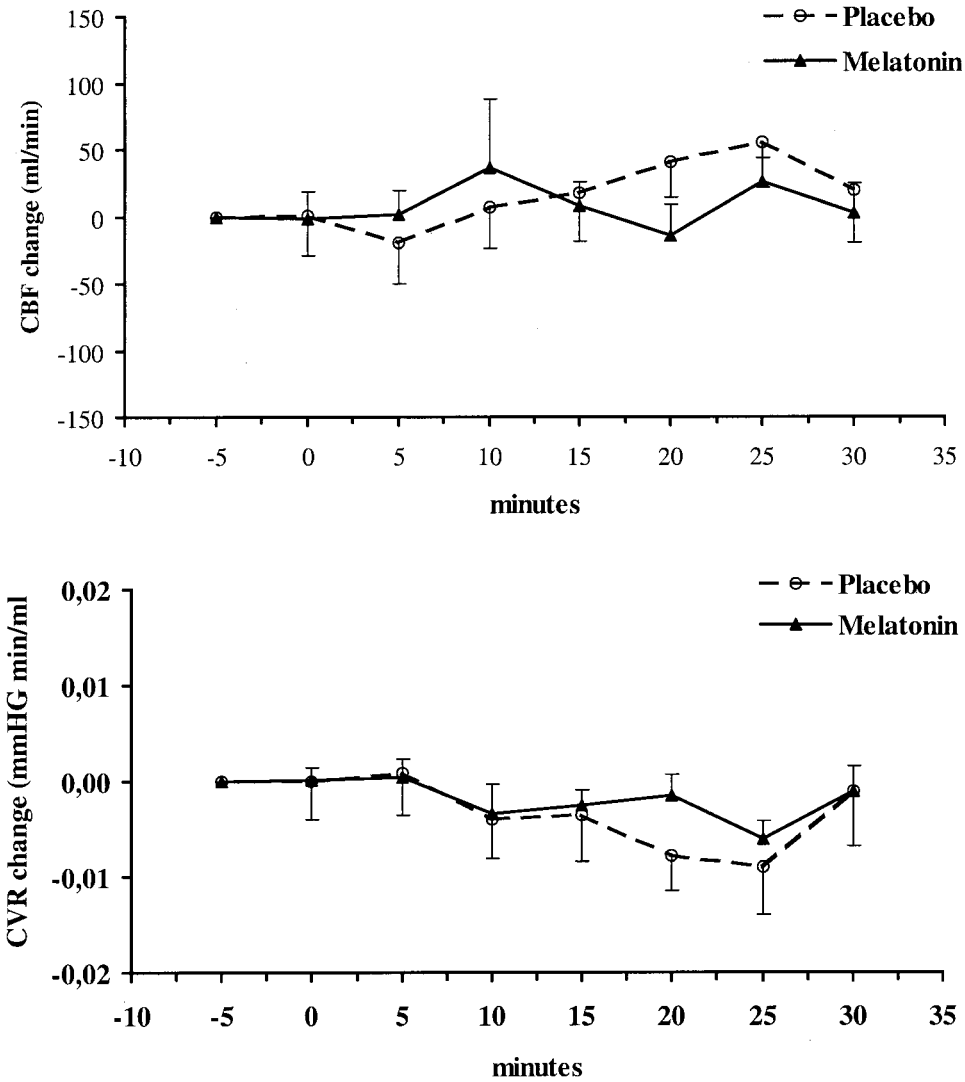

FIG. 1. Time course of the cerebrovascular variables. *Upper panel*, CBF (average ± SE). *Lower panel*, Cerebral vascular resistance. Values are expressed relative to the values assessed 5 min before infusion (time zero) of placebo (○) or melatonin (▲).

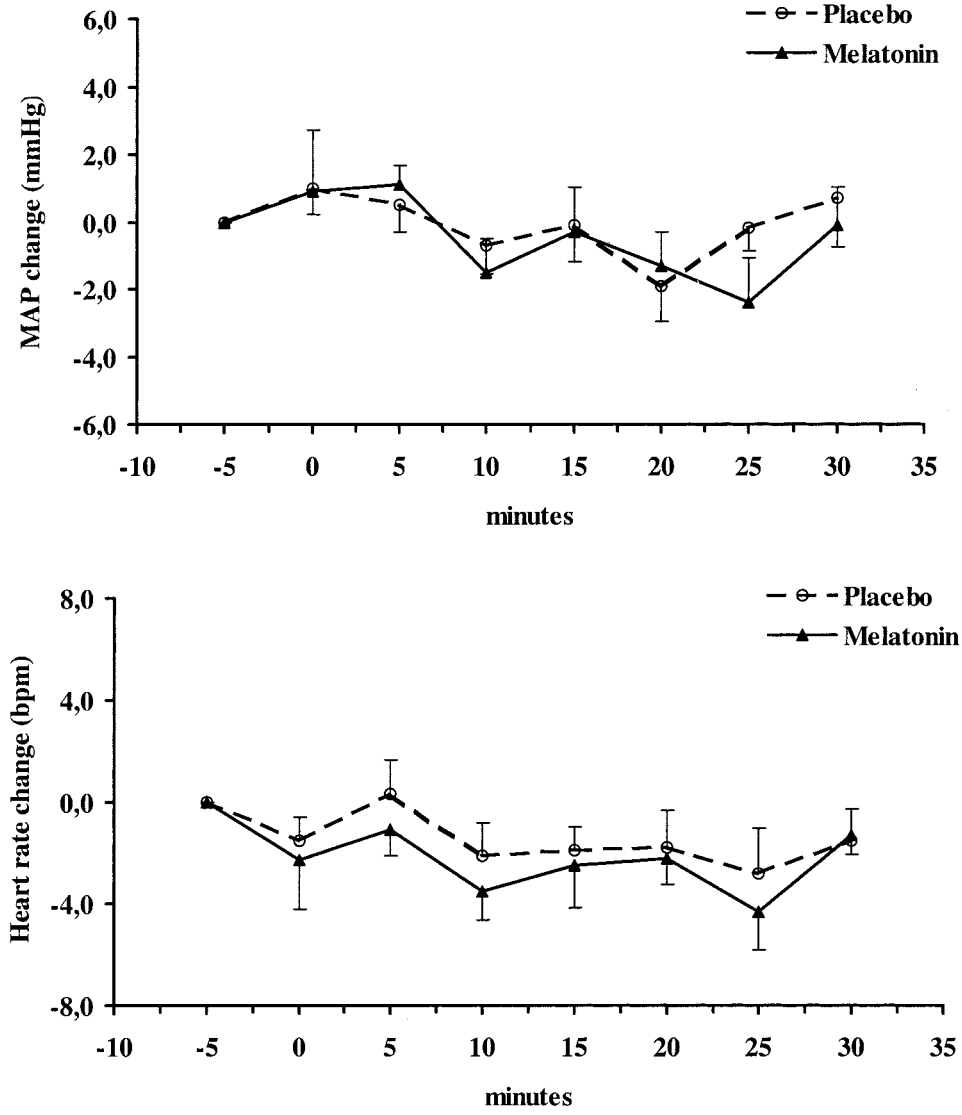

FIG. 2. Time course of the systemic vascular variables. *Upper panel*, MAP (average ± SE). *Lower panel*, Heart rate. MAP is expressed relative to the values assessed 5 min before the infusion (time zero) of placebo (○) or melatonin (▲). Values for heart rate represent changes in the median of the 5-min intervals and are expressed relative to the median assessed between 5 to 0 min before infusion (time zero) of placebo (○) or melatonin (▲).

ration (several hours) than the levels in our study (4). Furthermore, the induced plasma melatonin level was in agreement with previous studies reporting that melatonin has a very short half-life (17, 19), and that after iv administration a peak concentration is already reached after 3–5 min in the peripheral circulation and after 5–10 min in the brain (17, 20). Therefore, acute cerebrovascular effects would have been observed during the assessment interval of the present study, as confirmed by the peripheral effects. Our findings indicate that physiological levels of melatonin are unlikely to be involved in the reduced CBF that occurs during the night (8).

After melatonin infusion, we observed a comparable increase in PBF, measured with both PAT and DPG. Ideally, we should have used strain gauge venous occlusion plethysmography for determining the PBF. This technique, however, is not compatible with a magnetic environment of the MRI. The comparable increase in PBF as measured with our methods cross-validates the reliability of these noninvasive measurements and is in agreement with previous findings (5, 6).

We did not find an acute decrease in MAP after melatonin administration. This contrasts with previous work reporting a decrease after ingestion of 1 mg (4, 11), 3 mg (14), or 5 mg (21, 22) melatonin. As mentioned, even oral administration of 1 mg melatonin induced higher plasma levels than in our study. Therefore, it could be that melatonin in pharmacological concentrations induces a decrease in blood pressure, but our findings do not support an acute effect on blood pressure of a short-lived melatonin pulse with a low pharmacological concentration.

We found that melatonin did not affect plasma catecholamine levels. In contrast, it has been shown that norepinephrine levels decreased after ingestion of 2 mg (23) and 1 mg melatonin (4, 10). This also argues for dosage-dependent effects of melatonin.

The fact that administration of an iv bolus of melatonin induced peripheral vasodilation without changing CBF, MAP, or heart rate supports the idea that the acute effect of melatonin primarily acts via receptors in the peripheral vasculature rather than through altered autonomic activity (21), and that melatonin may exert its sleep-promoting effect at

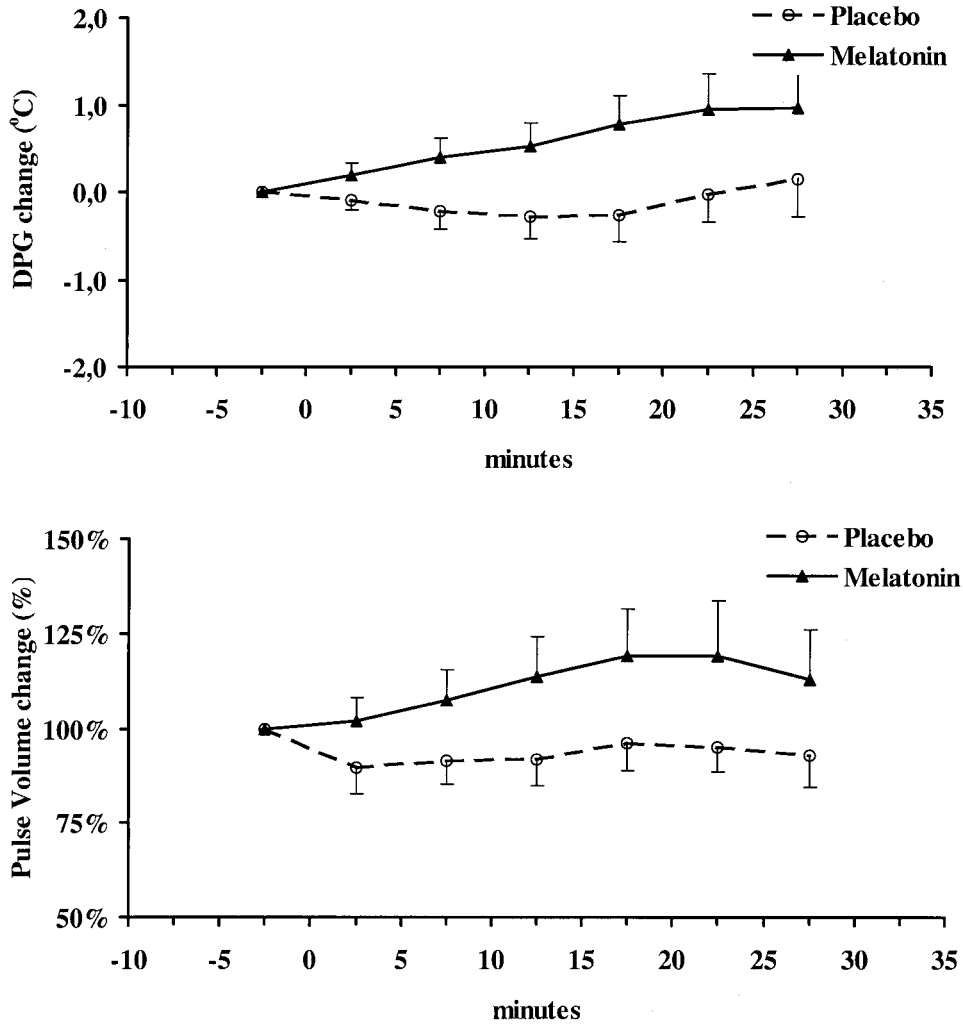

FIG. 3. Time course of the peripheral vascular variables. *Upper panel*, DPG (average ± SE). *Lower panel*, PAT photoplethysmography. Values represent changes in the median of the 5-min intervals and are expressed relative to the median assessed between 5 to 0 min before the infusion (time zero) of placebo (○) or melatonin (▲).

least in part through the induction of increased distal skin temperature (13). In isolated rat caudal artery segments, activation of the MT-1 receptor produced vasoconstriction, whereas at higher melatonin concentrations vasodilation was found, which appeared to be due to activation of the MT-2 receptor (24). Furthermore, *in vitro* studies with porcine vessels show that melatonin results in dose-dependent vasoconstriction in the coronary artery and dose-dependent vasodilation in the pulmonary artery (25). These findings suggest that the distribution of MT-1 and MT-2 receptors determines the net vascular response in various tissues. The distribution of vascular MT-1 and MT-2 receptors in humans is not known, and we cannot exclude the possibility that prolonged or high pharmacological plasma melatonin concentrations could alter CBF by affecting low affinity receptors.

In summary, our findings indicate that melatonin does not have an acute regulatory effect on CBF in humans, whereas it does increase PBF.

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