Melatonin improves cerebral circulation security margin in rats

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Régrigny, Olivier, Philippe Delagrange, Elizabeth Scalbert, Jeffrey Atkinson, and Isabelle Lartaud-Idjouadiene. Melatonin improves cerebral circulation security margin in rats. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H139–H144, 1998.—Because melatonin is a cerebral vasoconstrictor agent, we tested whether it could shift the lower limit of cerebral blood flow autoregulation to a lower pressure level, by improving the cerebrovascular dilatory reserve, and thus widen the security margin. Cerebral blood flow and cerebrovascular resistance were measured by hydrogen clearance in the frontal cortex of adult male Wistar rats. The cerebrovascular dilatory reserve was evaluated from the increase in the cerebral blood flow under hypercapnia. The lower limit of cerebral blood flow autoregulation was evaluated from the fall in cerebral blood flow following hypotensive hemorrhage. Rats received melatonin infusions of 60, 600, or 60,000 ng·kg⁻¹·h⁻¹, a vehicle infusion, or no infusion (n = 9 rats per group). Melatonin induced concentration-dependent cerebral vasoconstriction (up to 25% of the value for cerebrovascular resistance of the vehicle group). The increase in cerebrovascular resistance was accompanied by an improvement in the vasodilator response to hypercapnia (+50 to +100% vs. vehicle) and by a shift in the lower limit of cerebral blood flow autoregulation to a lower mean arterial blood pressure level (from 90 to 50 mmHg). Because melatonin had no effect on baseline mean arterial blood pressure, the decrease in the lower limit of cerebral blood flow autoregulation led to an improvement in the cerebrovascular security margin (from 17% in vehicle to 30, 55, and 55% in the low-, medium-, and high-dose melatonin groups, respectively). This improvement in the security margin suggests that melatonin could play an important role in the regulation of cerebral blood flow and may diminish the risk of hypoperfusion-induced cerebral ischemia.

MELATONIN INFLUENCES many physiological responses (9). Furthermore, melatonin has been shown to constrict large-diameter cerebral arteries in vitro (8). We therefore investigated the impact of melatonin on cerebrovascular resistance (CVR) reserve in vivo. We postulated that an increase in cerebral vasomotor tone would improve the cerebral vasodilatory capacity. The cerebrovascular dilatory reserve was investigated by measuring the effect of melatonin on the increase in cerebral blood flow (CBF) produced by hypercapnia, a challenge that is frequently used to test the cerebrovascular dilatory reserve (13, 16, 28). Finally, because the autoregulatory capacity of CBF is related to the cerebrovascular dilatory reserve (21), we investigated whether melatonin would modify the lower limit of CBF autoregulation. Experiments were performed in chronically instrumented, nonanesthetized, unrestrained rats that received an intravenous infusion of melatonin.

METHODS

Animals. Adult male Wistar rats (Ico:WI, IOPS AF/Han; Iffa-Credo, l’Arbresle, France; n = 45, weight = 516 ± 16 g) were randomized into control (no infusion), vehicle (infusion of solvent: NaCl 9‰ plus 1% of absolute ethanol), or treated groups (60, 600, or 60,000 ng·kg⁻¹·h⁻¹ melatonin) (n = 9 per group). The illumination schedule was lights on at 6:00 AM and lights off at 6:00 PM.

Baseline CBF, cerebrovascular resistance, blood gases, blood pH, arterial blood pressure, and heart rate. The methods used have been described in full elsewhere (11, 17). A brief summary will be given here.

After implantation of cortical electrodes (a platinum anode: diameter = 150 µm at coordinates A = +2 mm, L = +2 mm from the bregma; and a silver cathode: diameter = 200 µm at coordinates A = +2 mm, L = −2 mm from the bregma) under pentobarbital sodium anesthesia (60 mg/kg ip) on day 0, animals were allowed 12 days to recover. A polyethylene cannula (0.96/0.58 mm OD/ID; Merck Biotrol, Chennevieres, France) was placed via the femoral artery in the dorsal aorta, and a silicone catheter (1.94/0.64 mm OD/ID; Sigma Medical, Nanterre, France) was placed in the femoral vein under halothane (2%)-oxygen anesthesia. Animals were allowed 2 days to recover.

On the 14th day after implantation of the electrodes and 3 h after the lights came on, awake rats were placed in an air-tight cage (255 × 215 × 175 mm) through which air was passed at a flow rate of 10 l/min. The electrodes were connected to an amperometric circuit that applied +200 mV to the platinum cortical anode.

The femoral artery cannula was flushed and connected to a low-volume, strain-gauge transducer (Baxter, Bentley Laboratories). The femoral vein cannula was connected to an infusion pump (Bioblock Scientific, Paris, France) via a silicone tube and a plastic syringe (to avoid melatonin adsorption). Infusion of melatonin or vehicle at a rate of 0.254 ml/h was begun and continued throughout the experiment, i.e., 5 h.

A 60-min equilibration period allowed the plasma concentration of melatonin to equilibrate (results not shown). A mixture of air plus hydrogen (5%) was passed through the cage. The potential gradient between the two cortical electrodes produced a hydrogen oxidation current

\[ H_2 \rightarrow 2H^+ + 2e^- \]

Rats inhaled hydrogen until the oxidation current reached a stable level, showing that the cerebral cortex was saturated with hydrogen (5 min). At saturation, a blood sample (50 µl) was taken via the femoral artery cannula for the determination of pH, arterial PO₂ (PaO₂; mmHg), and arterial PCO₂ (PaCO₂; mmHg) (170 pH/Blood Gas Analyzer, Corning Medi-
melatonin by radioimmunoassay with the use of 2-[125I]iodo-
groups. Reacted to hypotensive hemorrhage in a similar fashion in all
within the next 2.5 h. The rise in blood pressure was similar
disconnecting the femoral artery cannula from the pressure
between volume and time of hemorrhage. Between treatment
groups was there any significant difference (two-way ANOVA)
was performed by 10 min. Blood pH, PaO2, PaCO2, arterial pressure, heart rate, and CBF were carried out at
15-min intervals. In none of the groups was there any
significant difference (one-way ANOVA) among any of the
time baseline measurements.

Cerebrovascular reactivity to hypercapnia. Cerebrovascu-
reactivity to carbon dioxide was determined 15 min after
the third and final measurement of baseline CBF by switch-
ing the gas mixture from air to air plus carbon dioxide (10%).
After 5 min of exposure to carbon dioxide, hydrogen was
added for a further 5 min. Blood pH, PaO2, PaCO2, arterial
pressure, heart rate, and CBF were then determined. Cerebro-
vascular reactivity to hypercapnia (ml·min⁻¹·100 g⁻¹·
mmHg⁻¹) is defined as the increase in CBF divided by the
increase in PaCO2. Rats next breathed air for 20 min to allow
blood pH, PaO2, PaCO2, arterial pressure, heart rate, and CBF
to return to baseline (data not shown).

Lower limit of CBF autoregulation. After a further 20-min
recovery period, i.e., 2.5 h after the beginning of infusion,
hemorrhage [20.8 ± 0.3 ml/kg in 10 ± 2 min; in none of the
groups was there any significant difference (two-way ANOVA)
between volume and time of hemorrhage] was performed by
disconnecting the femoral artery cannula from the pressure
transducer. Arterial pressure fell rapidly and then recovered
within the next 2.5 h. The rise in blood pressure was similar
in all groups (data not shown), suggesting that the neurohor-
monal compensatory reflexes of the extracranial circulation
reacted to hypotensive hemorrhage in a similar fashion in all
groups.

One milliliter of the blood removed during hypotensive
hemorrhage was used to measure the plasma concentration of
melatonin by radioimmunossay with the use of 2-[125I]iodo-
domelatonin (IM 215, Amersham, UK) and a melatonin
antibody (15940/16876, Guilford, UK).

While blood pressure rose during the 2.5-h period, 10
measurements of blood pH, PaO2, PaCO2, arterial pressure,
heart rate, and CBF were performed. For each treatment
group (control, vehicle, or melatonin), CBF (absolute values
and percent baseline), arterial pressure, heart rate, and blood
gas values were presented in the form used by Barry et al. (3).
Baseline values as well as values measured during the rise in
blood pressure were pooled and grouped by categories over
mean arterial blood pressure ranges of 20 mmHg. One-way
ANOVA within these different mean arterial blood pressure
ranges was performed for each treatment group. The lower
limit of CBF autoregulation (mmHg) was defined as the lower
limit of the lowest mean arterial blood pressure range in
which CBF was not significantly less than baseline CBF. The
security margin (%) was defined as [(baseline mean arterial
blood pressure – lower limit of CBF autoregulation) ×
100]/baseline mean arterial blood pressure (17).

Substances used. Melatonin was purchased from Sigma
Chemical (St. Louis, MO) and was dissolved in saline plus
absolute ethanol (1%). Doses are expressed in terms of base.
Halothane (Halothane Trofield) was purchased from Bé-
lamont (Paris, France). Oxygen, hydrogen, and carbon diox-
ide were purchased from Air Liquide (Nancy, France). Pento-
barbital sodium was purchased from Sanofi Santé Animale
(Liboir, France). All solutions were protected from light
to prevent the photodecomposition of melatonin.

Statistical analysis. Results are expressed as means ± SE.
The experimental protocol was designed for use of a one-way
ANOVA with the variable “treatment” (control, vehicle, or
melatonin at 60, 600, or 60,000 ng·kg⁻¹·h⁻¹). Significant
differences between means were determined using the Bonfer-
roni test. One-way ANOVA using the variable “mean arterial
blood pressure range” was performed separately for each
treatment group for the analysis of values obtained following
hypotensive hemorrhage. The probability level chosen was
P < 0.05.

RESULTS

Body weight decreased slightly (7 ± 2%) from day 0
to day 14 (516 ± 16 vs. 478 ± 10 g, n = 45, P = 0.016).
There were no differences among the groups at day 14.

Plasma concentration of melatonin. Plasma melato-
nin concentration was low in control and vehicle groups
(Table 1). The low-dose infusion rate (60 ng·kg⁻¹·h⁻¹) pro-
duced a sixfold increase in plasma melatonin concentra-
tion. A 10-fold increase in infusion rate doubled the plasma
melatonin concentration, and a further 100-fold increase
produced a 16-fold increase in plasma melatonin
concentration.

Baseline arterial blood pressure, heart rate, CBF,
cerebrovascular resistance, blood gases, and blood pH.
Arterial blood pressure (systolic, diastolic, mean, and
pulse), heart rate, pH, and blood gases were similar in
all groups (Table 1).

CBF and cerebrovascular resistance were similar in
vehicle and control groups (Table 1). Melatonin induced
a concentration-dependent diminution of CBF associ-
ated with an increase in cerebrovascular resistance
(Table 1). Cerebrovascular resistance was linearly re-
lated to the logarithm of the plasma concentration of
melatonin. Parameters of the linear regression were as
follows: slope = 0.16 ± 0.03 mmHg·min⁻¹·100 g·pg⁻¹,
intercept = 1.14 ± 0.09 mmHg·ml⁻¹·min⁻¹·100 g, n =
45, r² = 0.35, P < 0.05.

Cerebrovascular reactivity to hypercapnia. Carbon
dioxide inhalation produced a similar increase in PaCO2
in all groups (Table 2) and an increase in CBF of >50
to >170% (Table 2). Melatonin potentiated the vaso-
dilatory response to hypercapnia in a concentration-
dependent fashion as evidenced by the greater increase
in cerebrovascular reactivity during hypercapnia
(Table 2). There was a linear relationship between the
hypercapnia-induced decrease in cerebrovascular resis-
tance (y) and the logarithm of the melatonin plasma
concentration (x). Parameters of the linear regression
were as follows: slope = 0.22 ± 0.04 (mmHg·min⁻¹·100 g·pg⁻¹),
intercept = 0.14 ± 0.09 (mmHg·ml⁻¹·min⁻¹·100 g), n = 45, r² = 0.48, P < 0.01. Furthermore, there was
a significant correlation between the hypercapnia-
induced fall in cerebrovascular resistance (y) and bas-
line cerebrovascular resistance (x): slope = −0.98 ±
0.11 (no units), intercept = 0.82 ± 0.17 (mmHg·ml⁻¹·min⁻¹·100 g), n = 45, r² = 0.68, P < 0.01.

Carbon dioxide inhalation induced a similar degree of bradycardia in all groups but had no effect on mean or pulse arterial blood pressures (Table 2).

Lower limit of CBF autoregulation and security margin. After hypotensive hemorrhage in control and vehicle groups, CBF remained constant in the 110–129 and 90–109 mmHg ranges of mean arterial blood pressure but significantly decreased in the pressure ranges <90 mmHg (Table 3). The lower limit of CBF autoregulation was therefore 90 mmHg in these two groups. The security margin was 17% (Fig. 1 and Table 3).

In the melatonin-treated groups, the lower limit of CBF autoregulation was shifted to lower mean arterial blood pressure values (70 or 50 mmHg), and the security margin was improved (from 17% in the vehicle group to 33 and 55% in the melatonin-treated groups; Fig. 1 and Table 3).

Heart rate decreased in the lower blood pressure ranges in all treatment groups except the 600 ng·kg⁻¹·h⁻¹ melatonin group. PaO₂ increased and pH decreased as pressure fell below baseline values (Table 3). PaCO₂ decreased at lower blood pressures in the two higher melatonin dose groups.

### DISCUSSION

The present study shows that melatonin induces concentration-dependent cerebral vasodilation. This increase in vasodilatory tone accompanying the vasodilatory response to hypercapnia and shifts the lower limit of CBF autoregulation toward lower mean arterial blood pressure values. Because melatonin had no effect on baseline mean arterial blood pressure, the decrease in the lower limit leads to an improvement in the cerebrovascular security margin.

Such effects were obtained at physiological concentrations of melatonin. The plasma concentration of melatonin in control and vehicle groups was similar to the daytime value (10–50 pg/ml) (18). The plasma concentration of melatonin following infusion at a rate of 60 ng·kg⁻¹·h⁻¹ was similar to the nighttime plasma concentration of melatonin (100–300 pg/ml) (18).

Melatonin increases baseline cerebral vasodilatory tone (present results and Ref. 6). This amplifies the vasodilatory response to hypercapnia and shifts the lower limit of CBF autoregulation. This mechanism could involve large cerebral influx arteries and/or cerebral microvessels. The diameters of the large cerebral influx arteries are as important in the regulation of cerebral hemodynamics as the smaller cerebral efferent arteries.

### Table 2. Arterial blood pressure, heart rate, cerebral blood flow, cerebrovascular resistance, blood gases, and pH during CO₂ inhalation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vehicle</th>
<th>60</th>
<th>600</th>
<th>60,000</th>
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<tbody>
<tr>
<td><strong>n</strong></td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
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</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>120 ± 7</td>
<td>117 ± 3</td>
<td>110 ± 2</td>
<td>114 ± 5</td>
<td>117 ± 6</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>53 ± 4</td>
<td>53 ± 3</td>
<td>48 ± 5</td>
<td>53 ± 2</td>
<td>51 ± 2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>304 ± 20†</td>
<td>313 ± 16†</td>
<td>297 ± 17†</td>
<td>333 ± 14†</td>
<td>335 ± 17†</td>
</tr>
<tr>
<td>CBF, ml·min⁻¹·100 g⁻¹</td>
<td>134.1 ± 8.2†</td>
<td>121.2 ± 7.3†</td>
<td>142.0 ± 7.6†</td>
<td>143.2 ± 3.2†</td>
<td>168.2 ± 0.9†</td>
</tr>
<tr>
<td>CVR, mmHg·ml⁻¹·min⁻¹·100 g</td>
<td>0.96 ± 0.06†</td>
<td>0.99 ± 0.07†</td>
<td>0.79 ± 0.04†</td>
<td>0.85 ± 0.03†</td>
<td>0.75 ± 0.05†</td>
</tr>
<tr>
<td>RCO₂, ml·min⁻¹·100 g·mmHg⁻¹</td>
<td>1.9 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>2.8 ± 0.2*</td>
<td>3.6 ± 0.3*</td>
<td>3.8 ± 0.2*</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>112.2 ± 1.9†</td>
<td>108.9 ± 1.7†</td>
<td>107.6 ± 2.3†</td>
<td>109.5 ± 2.4†</td>
<td>112.2 ± 3.0†</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td>58.4 ± 2.3†</td>
<td>55.0 ± 1.9†</td>
<td>56.9 ± 1.3†</td>
<td>53.3 ± 1.5†</td>
<td>57.4 ± 2.1†</td>
</tr>
<tr>
<td>pH</td>
<td>7.241 ± 0.013†</td>
<td>7.245 ± 0.009†</td>
<td>7.230 ± 0.012†</td>
<td>7.239 ± 0.013†</td>
<td>7.221 ± 0.022†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. RCO₂, reactivity to CO₂ expressed as increase in CBF divided by increase in PaCO₂. *P < 0.05 vs. vehicle, one-way ANOVA and Bonferroni test. †P < 0.05 vs. baseline in same group (Table 1), one-way ANOVA and Bonferroni test.
CBF as those of the cerebral microvessels (12). It has been shown that melatonin has a direct vasoconstrictor effect on large cerebral influx arteries such as the anterior, middle, and posterior cerebral arteries (8). However, this is probably not the only factor involved. Indeed, Paulson and Waldemar (21) proposed that a change in the diameter of the large influx arteries would modify the response of the intracerebral arterioles downstream. Thus, if large cerebral arteries were constricted by melatonin, then vessels with small diameters would dilate and their vasodilatory responses to a fall in pressure would be lower. This would lead not to a change in baseline CBF but to a shift in the lower limit of CBF to a higher pressure level. Our results are in contradiction with this hypothesis, because we found that melatonin lowered baseline CBF and shifted the lower limit of CBF autoregulation to a lower pressure level. Furthermore, the cerebral vasoconstrictor activity to carbon dioxide, which is a measure of the dilatory capacity of cerebral microvessels (13, 16, 28), increased in a concentration-dependent manner following melatonin injection. All these observations lead us to suggest that melatonin constricts both large cerebral influx arteries and cerebral microvessels.

The vasoconstrictor effect of melatonin on large cerebral arteries has been reported to be direct and mediated by a Mel1 receptor (8). Information is lacking concerning the effect of melatonin on cerebral microvessels. However, the concentration-dependent nature of the cerebrovascular responses observed in the present study suggests that a receptor mechanism is involved. One can postulate that the beneficial effect of melatonin on the lower limit of CBF autoregulation should be antagonized by Mel1 antagonists (e.g., luzindole, S20928, or S20929; Refs. 7 and 26).

Indirect and nonspecific effects of melatonin cannot be ruled out. First, melatonin-induced vasoconstriction may partially be provoked by a reduction of neuronal metabolism (24) and cerebral glucose uptake (27) in the frontal cortex. Experiments using 2-deoxyglucose or...
other compounds that lower neuronal metabolism could clarify this matter. Second, melatonin-induced vasoconstriction may partially be related to an increase in the intracerebral concentration of serotonin (19). Third, although melatonin induces a decrease in intracerebral concentration of nitric oxide (10), this compound is not likely to be involved, because melatonin potentiates the vasodilatory response to hypercapnia, which is amplified by the release of nitric oxide (15).

Whatever the mechanism involved in the melatonin-induced vasoconstriction, our results may have clinical relevance in the area of stroke. The most important risk factor for stroke is age. After age 50, the risk of stroke doubles with each succeeding decade. Ischemic stroke is more common than hemorrhagic stroke in all age groups, particularly in the elderly group (in France) in which it comprises nearly 85% of all strokes (1). The increased frequency of ischemic stroke may be related to the fact that, with age, the lower limit of CBF autoregulation is shifted to a higher mean arterial blood pressure value (17, 23, 29). Furthermore, blood pressure lability increases with age, although mean arterial blood pressure tends to remain stable. Thus, in elderly normotensive subjects, the security margin is reduced. This decrease in the security margin, in association with the higher blood pressure lability, increases the risk of periodic cerebral hypoperfusion leading to cerebral ischemia. This could increase the frequency of cerebrovascular disorders in the elderly (4, 23). Our finding that acute melatonin infusion increases the vasodilatory reserve and improves the security margin suggests that melatonin, even if it induces a slight decrease in baseline CBF, may diminish the age-related increased risk of cerebral ischemia.

Along the same lines, it is interesting to note that melatonin secretion decreases with age (22) and that the incidence of stroke shows circadian (30) and circannual (20) cycles similar to those reported for plasma levels of melatonin. Finally, patients suffering from migraine have lower plasma melatonin concentrations (5) and a higher risk of stroke (25). Taken together, all these observations suggest that melatonin may have a cerebrovascular protective role and that this may involve an improvement in the security margin.

It should be noted that other factors may play a minor role in the response to hypotensive hemorrhage. \( P_{aO_2} \) increased, and \( pH \) decreased slightly, in all groups. Thus, although such changes may modify the lower limit of CBF autoregulation, the effect would be similar in all groups. \( P_{aCO_2} \) decreased to a greater extent in the lowest blood pressure ranges in the two higher dose groups. Given the linear relationship between CBF and \( P_{aCO_2} \) (14), the decrease in \( P_{aCO_2} \) does not explain why CBF is maintained until 50 mmHg in the two higher dose groups, but it may explain the fall in CBF at lower blood pressure values in these two groups.

In conclusion, acute melatonin administration increased the cerebrovascular dilatory capacity and thus improved the cerebrovascular security margin. This improvement in security margin suggests that melatonin may play an important role in the regulation of CBF. The relation of this to a possible involvement in hypoperfusion-induced cerebral ischemia in the elderly merits further study.

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