British Journal of Pharmacology (1999) 127, 1666–1670 
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# Effects of melatonin on rat pial arteriolar diameter *in vivo*

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**1** Based on our finding that melatonin decreased the lower limit of cerebral blood flow autoregulation in rat, we previously suggested that melatonin constricts cerebral arterioles. The goal of this study was to demonstrate this vasoconstrictor action and investigate the mechanisms involved.

**2** The effects of cumulative doses of melatonin ($10^{-10}$ to $10^{-6}$ M) were examined in cerebral arterioles (30–50 $\mu$M) of male Wistar rats using an open skull preparation. Cerebral arterioles were exposed to two doses of melatonin ($3\times10^{-9}$ and $3\times10^{-8}$ M) in the absence and presence of the $mt_1$ and/or $MT_2$ receptor antagonist, luzindole ($2\times10^{-6}$ M) and the $Ca^{2+}$-activated $K^+$ ($BK_{Ca}$) channel blocker, tetraethylammonium ($TEA^+$, $10^{-4}$ M). The effect of L-nitro arginine methyl ester (L-NAME, $10^{-8}$ M) was examined on arterioles after $TEA^+$ superfusion. Cerebral arterioles were also exposed to the $BK_{Ca}$ activator, NS1619 ($10^{-5}$ M), and to sodium nitroprusside (SNP, $10^{-8}$ M) in the absence and presence of melatonin ($3\times10^{-8}$ M).

**3** Melatonin induced a dose-dependent constriction with an $EC_{50}$ of $3.0\pm0.1$ nM and a maximal constriction of $-15\pm1$%. Luzindole abolished melatonin-induced vasoconstriction. $TEA^+$ induced significant vasoconstriction ($-10\pm2$%). No additional vasoconstriction was observed when melatonin was added to the aCSF in presence of $TEA^+$, whereas L-NAME still induced vasoconstriction ($-10\pm1$%). NS1619 induced vasodilatation ($+11\pm1$%) which was 50% less in presence of melatonin. Vasodilatation induced by SNP ($+12\pm2$%) was not diminished by melatonin.

**4** Melatonin directly constricts small diameter cerebral arterioles in rats. This vasoconstrictor effect is mediated by inhibition of $BK_{Ca}$ channels following activation of $mt_1$ and/or $MT_2$ receptors.

**Keywords:** Melatonin; cerebral arterioles; potassium channels; cerebral blood flow autoregulation

**Abbreviations:** aCSF, artificial cerebrospinal fluid; $BK_{Ca}$, $Ca^{2+}$ activated large-conductance $K^+$ channel; L-NAME, L-nitro arginine methyl ester; SNP, sodium nitroprusside; $TEA^+$, tetraethylammonium

## Introduction

We recently reported that melatonin increased the autoregulatory cerebrovascular dilatory capacity and improved the cerebrovascular security margin in rats (Régrigny *et al*., 1998), suggesting a potential protective effect of melatonin against hypotension-induced cerebral ischemia (Shuaib, 1992; Daffertshofer & Hennerici, 1995; Skoog, 1997). We suggested that melatonin increases the cerebrovascular dilatory capacity by constricting not only large cerebral influx arteries but also cerebral microvessels (Régrigny *et al*., 1998). Melatonin is known to constrict large-diameter cerebral arteries *in vitro*, following G protein-dependent inhibition of $Ca^{2+}$ activated large-conductance $K^+$ ($BK_{Ca}$) channels (Geary *et al*., 1997). In contrast, little is known of the effects of melatonin on small diameter cerebral arterioles which are involved in cerebral blood flow autoregulation.

The first goal of this study was to investigate the contractile effects of melatonin on small diameter (less than 50 $\mu$m) cerebral arterioles. The second goal was to determine whether the contractile effect of melatonin on small diameter cerebral arterioles was mediated by inhibition of $BK_{Ca}$ channels following activation of melatonin ($mt_1$ and/or $MT_2$) receptors, as previously described for large-diameter cerebral arteries (Geary *et al*., 1997). Experiments were performed using an open skull preparation in anaesthetized rats.

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## Methods

### *Animals and open skull preparation*

Experiments were performed on adult, male Wistar rats (Ico : WI, IOPS AF/Han; Iffa-Credo, l'Arbresle, France, body weight = 538 ± 22 g; 8–9 months). Animals were allowed free access to food and water, and housed at 24°C under controlled conditions of a 12 h light–dark cycle, lights on at 0600 and off at 1800). Experiments were performed in accordance with the guidelines of the European Union and the French Ministry of Agriculture.

Animals were anaesthetized with sodium pentobarbitone (60 mg $kg^{-1}$, i.p.) at 0900 h. A polyethylene cannula (Merck Biotrol, Chennevieres, France) was introduced into the left femoral artery, and connected to a low volume, strain-gauge transducer (Baxter, Bentley Laboratories, Europe) for measurement of blood pressure and heart rate. A polyethylene cannula was introduced into the right femoral artery to withdraw blood for measurement of arterial blood gases (pH, $PaO_2$, and $PaCO_2$, mmHg; 170 pH/Blood Gas Analyzer, Corning Medical, Medfield, U.S.A). A silicone catheter (Sigma Medical, Nanterre, France) was introduced into the left

femoral vein, and connected to a pump (Bioblock Scientific, Paris, France) for infusion of sodium pentobarbitone (at a rate of 0.25 ml $h^{-1}$; 20 mg $kg^{-1}$ $h^{-1}$) throughout the experiment to maintain anaesthesia. Animals were intubated and mechanically ventilated with room air (60 strokes $min^{-1}$; 10 ml $kg^{-1}$). Paralysis of skeletal muscle was obtained with gallamine triethiodide (20 mg $kg^{-1}$ i.v.) repeated every hour. Because the animals were paralyzed, the depth of anaesthesia was evaluated by applying pressure to the tail and observing changes in heart rate and blood pressure. Rectal temperature was maintained at 37–38°C with a heating pad. To comply with Home Office regulations, at the end of the experiment, animals were killed while under general anaesthesia.

### *Measurement of arteriolar diameter*

We measured the diameter of first order arterioles of the cerebrum (Harper & Bohlen, 1984) through an open skull preparation (Chillon *et al*., 1996; 1997). The head was placed in an adjustable head holder, and a 1 cm incision was made in the skin to expose the skull. The skin edges were retracted with sutures, and ports placed for inflow and outflow of artificial cerebrospinal fluid (aCSF). Craniotomy was performed over the left parietal cortex, and the dura was incised to expose cerebral vessels. The exposed brain was continuously suffused (3 ml $min^{-1}$) with aCSF, warmed to 37–38°C and equilibrated with a gas mixture of 5% $CO_2$–95% $N_2$. The composition of the aCSF was (mM) KCl, 3.0; $MgCl_2$, 0.6; $CaCl_2$, 1.5; NaCl, 131.9; $NaHCO_3$, 24.6; urea, 6.7; and glucose, 3.7 (Chillon *et al*., 1996; 1997).

Arterioles were monitored through a microscope (Stemi 2000-C, Carl Zeiss Jena GmbH, Jena, Germany) connected to a closed-circuit video system with a final magnification of $\times 400$. Images of arterioles were digitized using a video frame. Arteriolar diameter was measured from the digitized images with image analysis software (Saisam®, Microvision Instruments, Evry, France). The precision of this system is 0.5 $\mu$m (1 pixel = 0.5 $\mu$m).

### *Experimental protocols*

In a first experiment, we determined the effects of melatonin on cerebral arterioles. We examined latency and duration of the response induced by melatonin. For this we measured cerebral arteriolar diameter ($n=5$) every 5 min during perfusion with aCSF plus melatonin ($10^{-6}$ M). After 25 min, melatonin perfusion was stopped and we measured return in diameter to baseline. A cumulative concentration–response curve for melatonin ($n=8$) was constructed by adding increasing concentrations of melatonin in the range of $10^{-10}$ to $10^{-6}$ M. For each rat, a sigmoid curve fit was then calculated with the least squares method:

$$Y = \min + (\max - \min)/(1 + (10^{\log EC_{50}}/10^{\log X})^{slope})$$

where: Y = diameter variation ($\mu$m), X = dose of melatonin (M), min = minimum, max = maximum, $EC_{50}$ = the half-maximum effect, and slope = the slope of the curve.

The averages of min, max, $EC_{50}$ and slopes were then used to determine the average response curve. For the remainder of the experiments, two doses of melatonin, $3\times10^{-9}$ M ($EC_{50}$, see results) and $3\times10^{-8}$ M (inframaximal, see Results) were used.

In a second set of experiments, we explored the mechanisms by which melatonin induced contraction of cerebral arterioles. The effects of the melatonin receptor ($mt_1$ and $MT_2$) antagonist, luzindole (Dubocovich, 1995) ($n=7$), and the $BK_{Ca}$ channel blocker, tetraethylammonium (Brayden & Nelson, 1992) ($TEA^+$, $n=6$) on arteriolar vasoconstriction induced by melatonin were determined. Cerebral arterioles were exposed to two cumulative doses of melatonin ($3\times10^{-9}$ and $3\times10^{-8}$ M) prior to and after 20 min of superfusion of the cranial window with aCSF plus luzindole ($2\times10^{-6}$ M) or $TEA^+$ ($10^{-4}$ M). In order to verify that cerebral arterioles were still able to constrict after $TEA^+$, we repeated this experiment ($n=4$) with L-nitro arginine methyl ester (L-NAME, $10^{-8}$ M, a concentration which induced constriction of amplitude similar to that induced by melatonin, $3\times10^{-8}$ M) instead of melatonin. The effect of melatonin on the vasodilatation of cerebral arterioles induced by the $BK_{Ca}$ channel activator NS1619 (Holland *et al*., 1996) ($n=6$) was also assessed in the same rats. For this, cerebral arterioles were exposed to NS1619 ($10^{-5}$ M) prior to and 15 min after addition of melatonin at $3\times10^{-8}$ M in the aCSF. To verify that the effect of melatonin on NS1619-induced vasodilatation was specific, we repeated this experiment ($n=4$) with another vasodilatator, sodium nitroprusside (SNP, $10^{-8}$ M, a concentration which induced a similar degree of dilatation to that induced by NS1619). Finally, to verify that NS1619 acts *via* $BK_{Ca}$ channel in this preparation, we studied the effect of $TEA^+$ ($10^{-4}$ M) on NS1619-induced vasodilatation ($10^{-5}$ M, $n=6$). For this, cerebral arterioles were exposed to NS1619 prior to and 15 min after addition of $TEA^+$ in the aCSF.

All experiments started 30 min after completion of surgery which lasted about 2 h. All drugs, individually or in combination, were added to the aCSF at their final concentration and superfused in the cranial window until a stable response was obtained. For each drug concentration, diameters of cerebral arterioles, arterial blood pressure, heart rate, and blood gases were measured prior to the infusion of the drug and after stabilization of the diameter under drug infusion (about 20 min). Responses of cerebral arterioles to the various drugs were defined as the differences in diameters measured at the same point on the cerebral arteriole prior to and during drug infusion. Responses of cerebral arterioles were expressed either as absolute change in diameter ($\mu$m) or as percentage change, using the diameter measured prior to drug infusion as baseline. The cranial window was continuously superfused with aCSF between drug exposure for 30 min to washout drugs and allow the diameter of the cerebral arterioles to return to baseline.

### *Substances used*

Melatonin, luzindole, $TEA^+$, NS1619, SNP, L-NAME and gallamine triethiodide were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A). Melatonin, luzindole and NS1619 were dissolved in aCSF plus absolute ethanol (0.1% v $v^{-1}$) and protected from light. Control studies showed that the solvent had no effect on cerebral arteriolar diameter ($43\pm3$ *vs* baseline $45\pm2$ $\mu$m). Doses are expressed as base. Gases were purchased from Air Liquide (Nancy, France). Sodium pentobarbitone was purchased from Sanofi Santé Animale (Libourne, France). KCl, $MgCl_2$, $CaCl_2$, NaCl, $NaHCO_3$, urea and glucose were purchased from Merck KGaA (Darmstadt, Germany).

### *Statistical analysis*

Results are expressed as means ± s.e.mean. The experimental protocol was designed to use one-way analysis of variance (ANOVA). Significant differences between means were determined using the Bonferroni test. The probability level chosen was $P\leqslant0.05$.

## Results

### *Cumulative concentration-response curve for melatonin*

Melatonin induced dose-dependent constriction of cerebral arterioles (Figures 1, 2 and 3). The constriction of cerebral arterioles was maximal after 10 min perfusion with aCSF plus melatonin ($10^{-6}$ M). Thirty minutes after the end of the melatonin superfusion, cerebral arteriolar diameter returned to baseline values (Figure 3). The responses of cerebral arterioles to cumulative doses of melatonin were fit to a sigmoid curve ($Y=(-5.6\pm0.2)+((5.7\pm0.5)/(1+(10^{8.5\pm0.2}/10^{\log X}))^{1.0\pm0.1}$); $R^2=0.98$; $P<0.01$) with an $EC_{50}$ of $3.0\pm0.1$ nM, a maximum constriction of $-5.6\pm0.2$ $\mu$m ($-15\pm1\%$ *vs* baseline $38\pm2$ $\mu$m) and a slope of $1.0\pm0.1$ $\mu$m.mmol$^{-1}$.L.

### *Effects of luzindole on melatonin-induced constriction*

Luzindole ($2\times10^{-6}$ M), had no significant effect on cerebral arteriolar diameter ($46\pm2$ $\mu$m *vs* baseline diameter: $46\pm2$ $\mu$m) but abolished the constriction induced by melatonin (Figure 4).

### *Effects of $TEA^+$ on melatonin-induced constriction*

The $BK_{Ca}$ channel blocker, $TEA^+$ ($10^{-4}$ M), induced significant constriction of cerebral arterioles ($-10\pm2\%$ *vs* baseline $48\pm2$ $\mu$m) of a similar amplitude to that induced by melatonin ($3\times10^{-8}$ M : $-14\pm1\%$ *vs* baseline $47\pm3$ $\mu$m). No additional vasoconstriction was observed when melatonin ($3\times10^{-9}$ and $3\times10^{-8}$ M) was added to the aCSF in presence of $TEA^+$ (Figure 5).

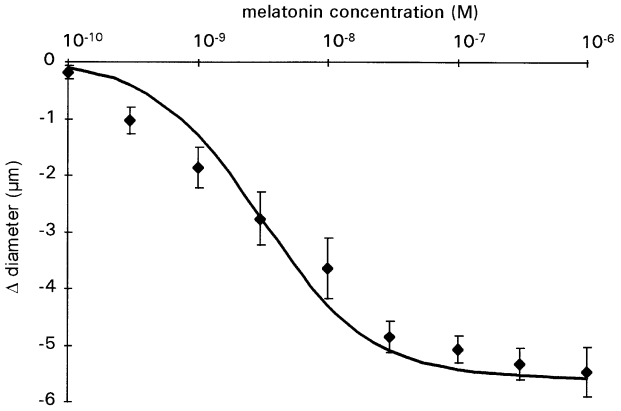


**Figure 1** Cumulative concentration-response curve for melatonin. Results are expressed as change in diameter from baseline. Values are means$\pm$s.e.mean; $n=8$. The sigmoid curve was calculated as the average of the individual curves obtained by least squares analysis of each data set (see Methods).

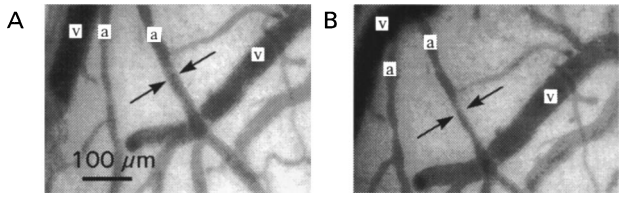


**Figure 2** Cerebral arterioles (a) and cerebral veins (v) superfused with aCSF (A) or with aCSF plus melatonin ($10^{-6}$ M; B). Arrows show the reduction in arteriolar diameter induced by melatonin.

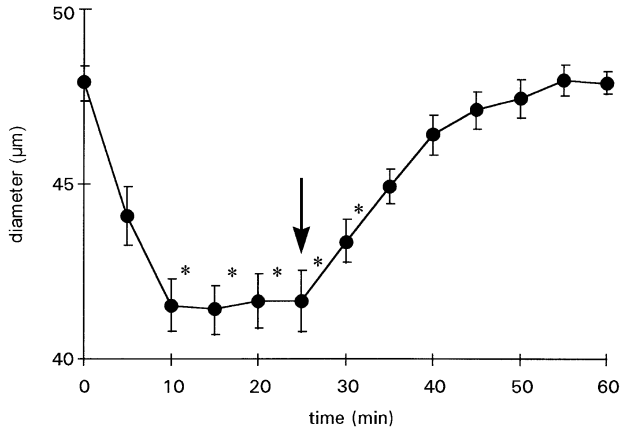


**Figure 3** Latency and duration effect of melatonin ($10^{-6}$ M) on cerebral arteriolar diameter. Arrow shows the end of the melatonin perfusion. Values are means$\pm$s.e.mean; $n=5$. *$P\leqslant0.05$ *vs* baseline.

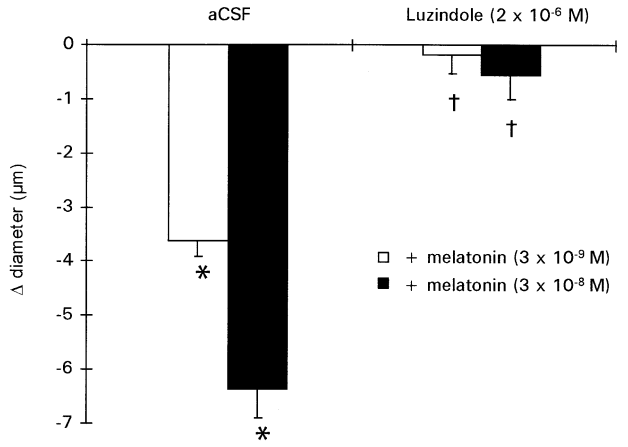


**Figure 4** Effect of melatonin ($3\times10^{-9}$ and $3\times10^{-8}$ M) prior to (aCSF) and after luzindole ($2\times10^{-6}$ M). Values are means$\pm$s.e.mean; $n=7$. *$P\leqslant0.05$ *vs* baseline. †$P\leqslant0.05$ *vs* responses induced by melatonin alone.

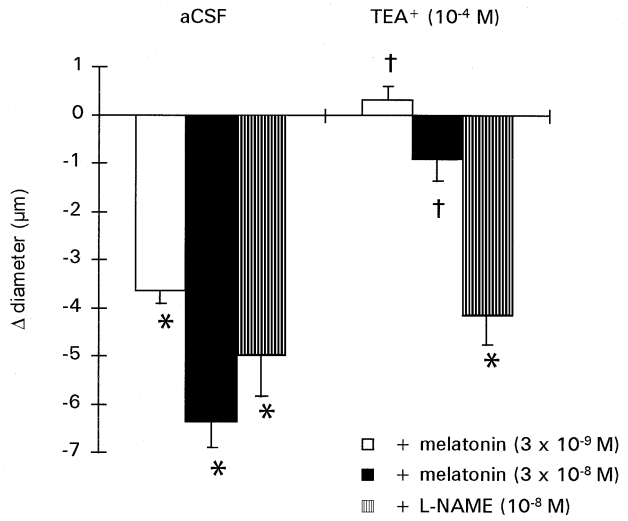


**Figure 5** Effect of melatonin ($3\times10^{-9}$ and $3\times10^{-8}$ M; $n=6$) or L-NAME ($10^{-8}$ M; $n=4$) prior to (aCSF) and after $TEA^+$ ($10^{-4}$ M). Values are means$\pm$s.e.mean. *$P\leqslant0.05$ *vs* baseline. †$P\leqslant0.05$ *vs* responses induced by melatonin alone.

*Effects of L-NAME on $TEA^+$-induced constriction*

The absence of vasoconstriction in cerebral arterioles preconstricted with $TEA^+$ was specific for melatonin as L-NAME ($10^{-8}$ M) in the presence of $TEA^+$ induced an additional, significant vasoconstrictor response (Figure 5).

*Effects of melatonin on NS1619- or SNP-induced dilatation of cerebral arterioles*

The $BK_{Ca}$ channel activator NS1619 ($10^{-5}$ M) induced a significant increase in cerebral arteriolar diameter ($+11\pm1\%$; *vs* baseline $45\pm3$ $\mu$m; Figure 6). Melatonin ($3\times10^{-8}$ M) induced significant constriction of cerebral arterioles ($-13\pm1\%$ *vs* baseline $45\pm3$ $\mu$m). NS1619-induced vasodilatation in the presence of melatonin was reduced by 50% ($+5\pm1\%$ *vs* baseline $39\pm3$ $\mu$m; Figure 6). $TEA^+$ significantly reduced vasodilatation induced by NS1619 ($+16\pm2\%$ vs $+8\pm1\%$).

SNP ($10^{-8}$ M) induced significant vasodilatation ($+12\pm2\%$; *vs* baseline $39\pm6$ $\mu$m; Figure 6). Melatonin ($3\times10^{-8}$ M) induced significant constriction of cerebral arterioles ($-12\pm1\%$ *vs* baseline $37\pm6$ $\mu$m). The vasodilator response to SNP in the presence of melatonin ($+17\pm4\%$ *vs* baseline $33\pm6$ $\mu$m) was equal to that obtained prior to melatonin superfusion (Figure 6).

Luzindole, melatonin, $TEA^+$, NS1619, L-NAME or SNP in the cranial window had no significant effect on systemic haemodynamics or blood gas parameters as heart rate ($357\pm14$ bpm), mean arterial blood pressure ($120\pm2$ mmHg), pH ($7.39\pm0.01$), $PaO_2$ ($96\pm3$ mmHg) and $PaCO_2$ ($36\pm1$ mmHg) did not change significantly throughout the experiment.

## Discussion

The present study shows that melatonin constricts small diameter cerebral arterioles *in situ* in anaesthetized rats. This effect appears to be mediated by the binding of melatonin on $mt_1$ and/or $MT_2$ receptors as the melatoninergic receptor ($mt_1$ and $MT_2$) antagonist, luzindole, abolished the response of cerebral arterioles to melatonin. Furthermore, our results suggest that the vasoconstriction induced by melatonin is due to inhibition of $BK_{Ca}$ channels as melatonin failed to elicit vasoconstriction when $BK_{Ca}$ channels were already inhibited by $TEA^+$ and reduced the vasodilatation induced by the $BK_{Ca}$ activator, NS1619.

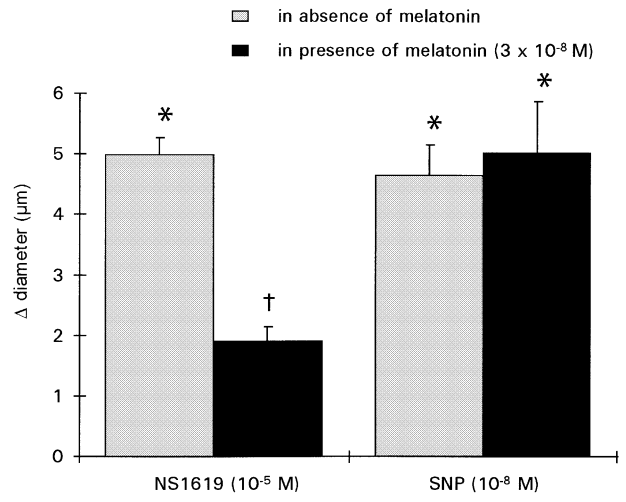


**Figure 6** Effect of melatonin ($3\times10^{-8}$ M) on the vasodilatory response to NS1619 ($10^{-5}$ M; $n=6$) or SNP ($10^{-8}$ M; $n=4$). Values are means$\pm$s.e.mean. *$P\leqslant0.05$ *vs* baseline. †$P\leqslant0.05$ *vs* responses induced by NS1619.

We previously suggested that melatonin constricts not only large cerebral influx arteries but also cerebral arterioles (Régrigny *et al*., 1998). A direct vasoconstrictor effect of melatonin on large-diameter cerebral arteries has already been established (Geary *et al*., 1997). Our hypothesis that melatonin would vasoconstrict small diameter cerebral arterioles was based on the following observations. First, the cerebrovascular response to carbon dioxide, which is a measure of the dilatory capacity of cerebral microvessels (Heistad *et al*., 1980; Wei *et al*., 1980; Jones *et al*., 1989), was increased in a concentration-dependent manner following melatonin infusion (Régrigny *et al*., 1998). Second, melatonin infusion shifted the lower limit of cerebral blood flow autoregulation towards a lower level of blood pressure (Régrigny *et al*., 1998), an effect that, according to the hypothesis of Paulson & Waldemar (1990), would only be observed if melatonin constricted not only large diameter cerebral arteries but also cerebral microvessels.

The present results confirm our hypothesis that melatonin constricts small diameter cerebral arterioles. In our study, melatonin constricted cerebral arterioles *in situ* with a potency ($EC_{50}=3.0\pm0.1$ nM) similar to that described for large diameter cerebral arteries *in vitro* pressurized to physiological levels ($EC_{50}=2.7$ nM) (Geary *et al*., 1997) or for pressurized tail arteries (0.7–3.2 nM) (Ting *et al*., 1997). Furthermore, the maximal decrease in diameter observed in our study ($-15\%$) was also similar to that measured in large-diameter cerebral ($10-17\%$) (Geary *et al*., 1997) or in tail arteries ($19-20\%$) (Ting *et al*., 1997). The response to melatonin was similar to that produced by maximum dose of another vasoconstrictor, endothelin ($22\pm5\%$) (Faraci, 1989).

The contractile effect of melatonin on cerebral arterioles was abolished by pretreatment with luzindole, a melatoninergic receptor antagonist (Krause & Dubocovich, 1991) at a concentration that has been reported to antagonize melatonin-induced vasoconstriction in isolated middle cerebral arteries (Geary *et al*., 1997). This result supports the hypothesis that, as in large-diameter cerebral arteries (Geary *et al*., 1997), melatonin acts on a vascular $mt_1$ and/or $MT_2$ receptor. However, as melatonin is known to be an anti-oxidant (Reiter, 1997) and as free radicals are known to dilate blood vessels *via* effects on potassium channels (Archer *et al*., 1994) we cannot rule out the possibility that such a mechanism is at least partly responsible for melatonin-induced constriction in our experiments.

In large diameter cerebral arteries, such as the middle cerebral artery, melatonin induces vasoconstriction by blockade of receptor-coupled $BK_{Ca}$ (Geary *et al*., 1997). The present results suggest that the same mechanism operates in arterioles. Melatonin failed to elicit further vasoconstriction in cerebral arterioles preconstricted with the $BK_{Ca}$ channel blocker $TEA^+$, suggesting that the two drugs act *via* the same pathway. This was confirmed when we examined the effect of a vasoconstrictor with a different mechanism of action, L-NAME, on cerebral arterioles preconstricted with $TEA^+$. L-NAME significantly constricted cerebral arterioles preconstricted with $TEA^+$. An additional indication that melatonin-induced vasoconstriction in cerebral arterioles is mediated by the $BK_{Ca}$ channels is given by the results of the experiment using NS1619. NS1619, a $BK_{Ca}$ channel opener, induced vasodilatation in cerebral arterioles and this was antagonized by

melatonin and $TEA^+$. This effect of melatonin appears to be specific for the $BK_{Ca}$ channel opener, NS1619, as melatonin failed to antagonize vasodilatation of cerebral arterioles induced by a vasodilatory agent with a different mechanism of action, SNP. NS1619 has been shown to inhibit L-type $Ca^{2+}$ channels and voltage-activated $K^+$ channels (Holland *et al*., 1996), however the fact that $TEA^+$ inhibits NS1619 vasodilatation is consistent with an effect of NS1619 on $BK_{Ca}$ channels in our preparation.

### *Implications of our findings*

In conclusion, the present results show that melatonin vasoconstricts small diameter cerebral arterioles. This direct constrictor effect of melatonin in small diameter cerebral arterioles appears to be mediated by melatoninergic ($mt_1$ and/or $MT_2$) receptors. The results also suggest that, as observed in large-diameter cerebral arteries (Geary *et al*., 1997), vasoconstriction induced by activation of the receptor is mediated by inhibition of $BK_{Ca}$ channels. This direct vasoconstrictor effect of melatonin observed *in vivo* on cerebral arterioles, together with the vasoconstrictor effect of melatonin observed *in vitro* on large-diameter cerebral arteries (Geary *et al*., 1997), may explain the increase in the cerebrovascular dilatory capacity and the decrease of the lower limit of cerebral blood flow autoregulation that we have previously described (Régrigny *et al*., 1998).

This study was funded by grants from the French Ministry of Education and Research (EA/JE2166, Paris, France), the Regional Development Committee (Metz, France), the Greater Nancy Urban Council (Nancy, France), the Henri Poincaré University (Nancy, France) and Institut de Recherches Internationales Servier (Courbevoie, France). Part of these results was presented at the Winter Meeting of the British Pharmacological Society, Brighton, U.K. (Chillon *et al*., 1999).

## References

ARCHER, S.L., HUANG, J.M., HAMPL, V., NELSON, D.P., SHULTZ, P.J. & WEIR, E.K. (1994). Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cGMP-dependent protein kinase. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 7583–7587.

BRAYDEN, J.E. & NELSON, M.T. (1992). Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science,* **256,** 532–535.

CHILLON, J.M., GHONEIM, S. & BAUMBACH, G.L. (1997). Effects of chronic nitric oxide synthase inhibition on cerebral arterioles in rats. *Hypertension,* **30,** 1097–1104.

CHILLON, J.M., HEISTAD, D.D. & BAUMBACH, G.L. (1996). Effects of endothelin receptor inhibition on cerebral arterioles in hypertensive rats. *Hypertension,* **27,** 794–798.

CHILLON, J.M., RÉGRIGNY, O., LARTAUD-IDJOUADIENE, I., DELAGRNAGE, P., SCALBERT, E. & ATKINSON, J. (1999). Melatonin constricts rat cerebral arterioles in vivo by blocking $Ca^{2+}$-activated $K^+$ ($BK_{Ca}$) channels. *Br. J. Pharmacol.,* **126,** 34P.

DAFFERTSHOFER, M. & HENNERICI, M. (1995). Cerebrovascular regulation and vasoneuronal coupling. *J. Clin. Ultrasound,* **23,** 125–138.

DUBOCOVICH, M.L. (1995). Melatonin receptors: are there multiple subtypes? *Trends Pharmacol. Sci.,* **16,** 50–56.

FARACI, F.M. (1989). Effects of endothelin and vasopressin on cerebral blood vessels. *Am. J. Physiol.,* **257,** H799–H803.

GEARY, G.G., KRAUSE, D.N. & DUCKLES, S.P. (1997). Melatonin directly constricts rat cerebral arteries through modulation of potassium channels. *Am. J. Physiol.,* **273,** H1530–H1536.

HARPER, S.L. & BOHLEN, H.G. (1984). Microvascular adaptation in the cerebral cortex of adult spontaneously hypertensive rats. *Hypertension,* **6,** 408–419.

HEISTAD, D.D., MARCUS, M.L., PIEGORS, D.J. & ARMSTRONG, M.L. (1980). Regulation of cerebral blood flow in atherosclerotic monkeys. *Am. J. Physiol.,* **239,** H539–H544.

HOLLAND, M., LANGTON, P.D., STANDEN, N.B. & BOYLE, J.P. (1996). Effects of the $BK_{Ca}$ channel activator, NS1619, on rat cerebral artery smooth muscle. *Br. J. Pharmacol.,* **117,** 119–129.

JONES, S.C., BOSE, B., FURLAN, A.J., FRIEL, H.T., EASLEY, K.A., MEREDITH, M.P. & LITTLE, J.R. (1989). $CO_2$ reactivity and heterogeneity of cerebral blood flow in ischemic, border zone, and normal cortex. *Am. J. Physiol.,* **257,** H473–H482.

KRAUSE, D.N. & DUBOCOVICH, M.L. (1991). Melatonin receptors. *Ann. Rev. Pharmacol. Toxicol.,* **31,** 549–568.

PAULSON, O.B. & WALDEMAR, G. (1990). Inhibition of the renin angiotensin system and the autoregulation of cerebral circulation. In *Coronary and Cerebrovascular Effects of Antihypertensive Drugs*. ed. Atkinson, J., Capdeville, C. & Zannad, F. pp. 129–144. London: Transmedica.

RÉGRIGNY, O., DELAGRANGE, P., SCALBERT, E., ATKINSON, J. & LARTAUD-IDJOUADIENE, I. (1998). Melatonin improves cerebral circulation security margin in rats. *Am. J. Physiol.,* **275,** H139–H144.

REITER, R.J. (1997). Antioxidant actions of melatonin. *Advances in Pharmacology,* **38,** 103–117.

SHUAIB, A. (1992). Alteration of blood pressure regulation and cerebrovascular disorders in the elderly. *Cerebrovasc. Brain Met. Rev.,* **4,** 329–345.

SKOOG, I. (1997). The relationship between blood pressure and dementia: a review. *Biomed. Pharmacother.,* **51,** 367–375.

TING, K.N., DUNN, W.R., DAVIES, D.J., SUGDEN, D., DELAGRANGE, P., GUARDIOLA-LEMAÎTRE, B., SCALBERT, E. & WILSON, V.G. (1997). Studies on the vasoconstrictor action of melatonin and putative melatonin receptor ligands in the tail artery of juvenile Wistar rats. *Br. J. Pharmacol.,* **122,** 1299–1306.

WEI, E.P., KONTOS, H.A. & PATTERSON, J.L.J. (1980). Dependence of pial arteriolar response to hypercapnia on vessel size. *Am. J. Physiol.,* **238,** H697–H703.

*(Received, February 2, 1999*
*Accepted, May 11, 1999)*