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European Journal of Pharmacology 417 (2001) 125–129

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# Weak vasoconstrictor activity of melatonin in human umbilical artery: relation to nitric oxide-scavenging action

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Received 5 October 2000; received in revised form 23 January 2001; accepted 26 January 2001

**Abstract**

We evaluated the nitric oxide (NO)-scavenging property of melatonin, demonstrated in a recent in vitro study, on vascular reactivity in the human umbilical artery. Helical sections of human umbilical artery were prepared following elective Cesarean deliveries near term. Changes in maximal tension induced by prostaglandin $F_{2\alpha}$($5 \times 10^{-5}$ M) were measured in artery sections with an intact endothelium. Melatonin at concentrations higher than $10^{-6}$ M increased prostaglandin $F_{2\alpha}$-induced vascular tension. The vasospastic effect of melatonin was much less than that of L-$N^G$-monomethylarginine (L-NMA, $2 \times 10^{-4}$ M), an inhibitor of NO synthesis ($2.8 \pm 1.4\%$, $9.1 \pm 1.7\%$, $16.5 \pm 2.5\%$, and $29.6 \pm 5.9\%$ of the L-NMA effect at melatonin concentrations of $10^{-8}$, $10^{-7}$, $10^{-6}$, and $10^{-5}$ M, respectively). Removal of the endothelium significantly reduced the vasoconstrictive effect of melatonin. Treatment with L-NMA ($2 \times 10^{-4}$ M) prior to addition of prostaglandin $F_{2\alpha}$ also significantly reduced the vasoconstrictive effect of melatonin ($10^{-5}$ M). Treatments with melatonin ($10^{-5}$ M) did not affect calcium ionophore A 23187-induced relaxation or 5-hydroxytryptamine-induced constriction. The findings indicate that melatonin may potentiate vascular tension in human umbilical artery by scavenging endogenous endothelial NO, but not by inhibiting NO synthesis. However, the NO-scavenging vasoconstrictive effect of melatonin may be negligible at physiologic concentrations and very weak at pharmacologic concentrations below $10^{-7}$ M. 

*Keywords:* Melatonin; Vasospasm; Umbilical artery; Nitric oxide (NO)

## 1. Introduction

Melatonin, which is secreted by the pineal gland, contributes to the regulation of biologic rhythms and neuroendocrine functions, including those of the reproductive system and the adrenal glands (Reiter, 1991). Increasing attention has turned to the antioxidant properties of melatonin, which is a powerful scavenger of oxygen-free radicals, including hydroxyl radical, singlet molecular oxygen, peroxynitrite ion (Tan et al., 1993, 1998; Reiter et al., 1999) and possibly also peroxyl radicals (Pieri et al., 1994).

Although the effect of exogenous melatonin on human cardiovascular responses is largely unknown, some clinical investigations suggest that melatonin may have an influence. Administration of melatonin to healthy young women was found to reduce blood pressure and the pulsatility index, which is thought to directly represent impedence to blood flow, at 90 min following a 1-mg oral dose (Cagnacci et al., 1997, 1998). Long-term administration of melatonin (2 mg/day intranasally for 1 week) to patients with essential hypertension significantly reduced blood pressure (Birau et al., 1981).

Nitric oxide (NO), a small, diffusible messenger molecule synthesized from the amino acid L-arginine by nitric oxide synthase (NOS), originally was identified as the principal endothelium-derived vascular relaxation factor. Nowadays, NO also is recognized as a neurotransmitter in the central nervous system (Moncada et al., 1991). Pozo et al. (1994) have shown that rat cerebellar NOS activity is inhibited by physiologic concentrations of melatonin. The inhibition is dose dependent and is coupled to inhibition of cyclic GMP production activated by L-arginine. Furthermore, a recent report indicated that melatonin and its precursors scavenge NO in vitro (Noda et al., 1999). However, whether melatonin can inhibit NOS activity in the vascular endothelium has not been elucidated.

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0014-2999/01/$ - see front matter 
PII: S0014-2999(01)00802-0

Previously, we demonstrated that hydrogen peroxide ($H_2O_2$) and oxidized low-density lipoprotein (LDL) potentiate vascular tension in the human umbilical artery by suppressing the endothelial synthesis of NO (Okatani et al., 1997, 2000). Melatonin significantly suppressed the vasospastic effect of $H_2O_2$ and oxidized LDL, possibly by scavenging hydroxyl radicals (Okatani et al., 1997, 2000). In our laboratory, we recently evaluated the possibility of therapeutic use of melatonin in pre-eclampsia and fetal hypoxia, where excessive oxygen-free radical production is suspected (Okatani et al., 1997; Wakatsuki and Okatani, 2000). However, little is known about the direct effect of melatonin on vascular tone in the human umbilical artery. The purpose of the present study was to evaluate the short-term effect of melatonin on vascular tension as related to NO-scavenging ability and NOS activity in this vessel.

## 2. Materials and methods

### 2.1. Drugs

L-$N^G$-monomethylarginine (L-NMA), 5-hydroxytryptamine (5-HT), prostaglandin $F_{2\alpha}$ and calcium ionophore A 23187 were purchased from Sigma (St. Louis, MO, USA). Melatonin, purchased from Aldrich Chemical (Milwaukee, WI, USA), was dissolved in a minimal volume of absolute ethanol, diluted to $10^{-5}$ M with incubation medium (see below), and stored at −60°C. The concentration of ethanol in the final solution was lower than 0.5%.

### 2.2. Preparations

Umbilical cords were obtained from healthy Japanese women immediately after elective Cesarean section between 37 and 39 weeks of gestation. Segments 15 to 20 cm in length were cut from the umbilical cord between the placenta and point of cord transaction, and the umbilical artery was sectioned helically to provide strips 3- to 4-mm wide and 25-mm long. When endothelium-free sections were required, endothelium removal was accomplished by gently rubbing the luminal surface with a paper towel moistened with incubation solution as previously described (Okatani et al., 2000). Sections of umbilical artery were suspended in an organ bath filled with modified Krebs–Ringer's solution at 37°C (17 ml, pH 7.4) containing (in mM): sodium chloride (117.2); potassium chloride (5.9); calcium chloride dihydrate (2.0); magnesium sulfate (0.6); sodium bicarbonate (25.0); sodium phosphate (1.2); and glucose (11.1). The bath was continuously aerated with 95% $O_2$/5% $CO_2$. All experiments were performed 6 h after collection of specimens. Each section was attached by metal hooks to a force-displacement transducer (Nihon Kohden, Tokyo, Japan) and a tension adjustment device. Sections were allowed to equilibrate for 30 min at a resting tension of 2 g. Load tension was maintained and adjusted periodically throughout the period of incubation and during the experiment. Each experiment was preceded by two sequential challenges with KCl (60 mM). Artery sections were treated as described, and isometric contractile activity was recorded with a polygraph (Nihon Kohden).

### 2.3. Experimental protocol

#### 2.3.1. Study 1

To evaluate the effect of melatonin on vascular tension, umbilical artery sections with endothelium from 10 different preparations were exposed to prostaglandin $F_{2\alpha}$ ($5 \times 10^{-5}$ M) to induce constriction. Melatonin ($10^{-8}$ to $10^{-5}$ M) or L-NMA ($2 \times 10^{-4}$ M), an inhibitor of NO synthesis, was added after the maximal vasoconstrictive response to prostaglandin $F_{2\alpha}$ had been achieved.

#### 2.3.2. Study 2

To determine whether the vasoconstrictive effect of melatonin is dependent on the endothelium, umbilical artery sections with or without endothelium from eight different preparations were exposed to prostaglandin $F_{2\alpha}$ ($5 \times 10^{-5}$ M). Melatonin ($10^{-5}$ M) was added after a maximal vasoconstrictive response to prostaglandin $F_{2\alpha}$ had been achieved.

#### 2.3.3. Study 3

To determine whether the vasoconstrictive effect of melatonin is mediated by NO, umbilical artery sections with an intact endothelium from 10 different preparations were preincubated with L-NMA ($2 \times 10^{-4}$ M) for 15 min prior to addition of prostaglandin $F_{2\alpha}$ ($5 \times 10^{-5}$ M). Melatonin ($10^{-5}$ M) was added after the maximal vasoconstrictive response to prostaglandin $F_{2\alpha}$.

#### 2.3.4. Study 4

To determine the effect of melatonin on NO-induced relaxation, umbilical artery sections from 10 different preparations were preincubated with or without melatonin ($10^{-5}$ M) for 20 min. Calcium ionophore A 23187 ($10^{-5}$ M) was added following a maximal vasoconstrictive response to prostaglandin $F_{2\alpha}$ ($5 \times 10^{-5}$ M).

#### 2.3.5. Study 5

The effect of melatonin on 5-HT-induced constriction was evaluated by preincubating umbilical artery sections from 10 different preparations for 20 min with melatonin ( $10^{-5}$ M), after which 5-HT was added in a cumulative manner ( final concentrations; 1 to 1000 ng/ml).

### 2.4. Calculations and statistical analysis

Data are expressed as the means ± S.E. Changes in tension are expressed as percentages of maximal prosta-

glandin $F_{2\alpha}$- or KCl-induced constriction. Patterns of melatonin- or 5-HT-induced constriction were analyzed by one-way of analysis of variance or two-way analysis of variance with repeated measure. Multiple comparison analysis was performed with Scheffe's test. Differences in mean values between two groups were analyzed with Welch's test. A level of $P < 0.05$ was considered statistically significant.

## 3. Results

The tracings reproduced in Fig. 1 show the effect of melatonin ($10^{-5}$ M) and L-NMA on isometric changes during stable constriction evoked by prostaglandin $F_{2\alpha}$. Melatonin increased vascular tension at concentrations higher than $10^{-6}$ M (Fig. 2). However, the vasospastic effect of melatonin was much less than that induced by L-NMA, with melatonin at $10^{-5}$ M producing only $29.6 \pm 5.9\%$ of the effect of L-NMA at $2 \times 10^{-4}$ M. The vasospastic effect of melatonin at concentrations below $10^{-7}$ M was less than 10% of that of L-NMA at $\times 10^{-4}$ M ($2.8 \pm 1.4\%$ for $10^{-8}$ melatonin, $9.1 \pm 1.7\%$ for $10^{-7}$ M).

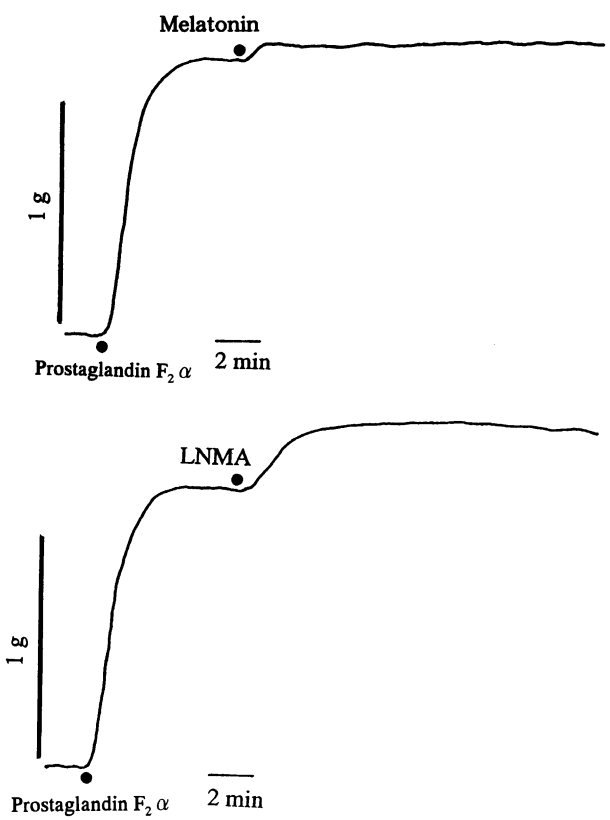


Fig. 1. Representative tracings showing effects of melatonin ($10^{-5}$ M, upper tracing) and L-$N^G$-monomethylarginine (L-NMA, $2 \times 10^{-4}$ M, lower tracing) on isometric tension changes during stable constriction evoked by prostaglandin $F_{2a}$ ($5 \times 10^{-5}$ M).

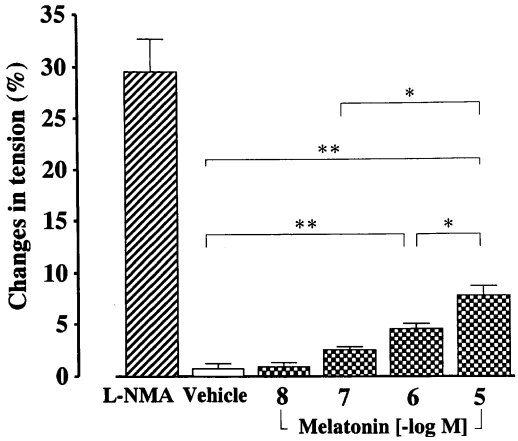


Fig. 2. Bar graphs showing induction of tension by either L-$N^G$-monomethylarginine (L-NMA, $2 \times 10^{-4}$ M; diagonally hatched column) or melatonin ($10^{-8}$ to $10^{-5}$ M, checkered columns) in human umbilical artery sections with endothelium. Open column indicates the vehicle-treated control group. Data are expressed as the means ± S.E. Changes in tension are expressed as percentages of the prostaglandin $F_{2\alpha}$($5 \times 10^{-5}$ M)-induced maximal constriction. $^{*}P < 0.01$, $^{**}P < 0.001$. The $P$ values were obtained by Scheffe's test.

The vasospastic effect of melatonin was significantly reduced by removal of the endothelium from the artery sections ($P < 0.001$, Fig. 3). L-NMA treatment prior to $PGF_{2\alpha}$-induced constriction also significantly suppressed the vasospastic effect of melatonin ($P < 0.001$, Fig. 4). No significant difference in maximum relaxation induced by calcium ionophore A 23187 was found between the preparations exposed ($66.4 \pm 5.1\%$) or not exposed ($63.6 \pm 4.2\%$) to melatonin. No significant difference in 5-HT-in-

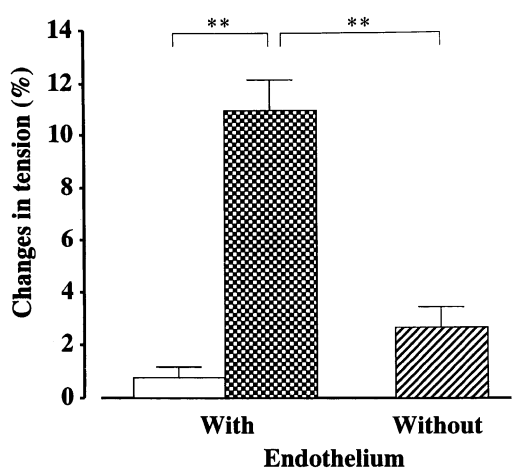


Fig. 3. Bar graphs showing induction of tension by melatonin ($10^{-5}$ M) in human umbilical artery sections with (checkered column) or without endothelium (diagonally hatched column). Open column indicates the vehicle-treated control group. Data are expressed as the means ± S.E. Changes in tension are expressed as percentages of the prostaglandin $F_{2\alpha}$ ($5 \times 10^{-5}$ M)-induced maximal constriction. $^{**}P < 0.001$. The $P$ value was obtained by Welch's test.

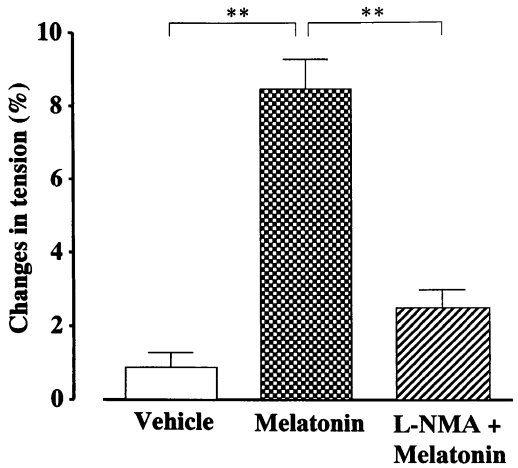


Fig. 4. Bar graphs showing induction of tension by melatonin ($10^{-5}$ M) in human umbilical artery sections with endothelium. Artery sections were pretreated with (diagonally hatched column) or without (checkered column) L-$N^G$-monomethylarginine (L-NMA, $2\times10^{-4}$ M). Open column indicates the vehicle-treated control group. Data are expressed as the means ± S.E. Changes in tension are expressed as percentages of the maximal prostaglandin $F_{2\alpha}$($5\times10^{-5}$ M)-induced constriction. $^{**}P <$ 0.001. The $P$ value was obtained by Welch's test.

duced constriction was found between the preparations with or without MLT treatment (data not shown).

## 4. Discussion

The present study demonstrated that melatonin concentrations higher than $10^{-6}$ M potentiated vascular tension in preconstricted helical strips of human umbilical artery. This vasoconstrictive action of melatonin is largely dependent on the endothelium, since removal of this layer significantly suppressed the potentiation of vascular tension induced by melatonin. Earlier studies also demonstrated that melatonin produces concentration-dependent contraction of preconstricted helical strips of isolated rat caudal artery (Viswanathan et al., 1990) and preconstricted pig coronary artery (Weekley, 1993). In the present study, we did not examine the direct effect of low or high melatonin concentrations on resting tone, but Evans et al. (1992) have demonstrated a direct vasoconstrictive action of melatonin in resting pressurized segments of caudal artery isolated from juvenile rats.

In the absence of autonomic innervation, the circulation in the human umbilical vessels is regulated by autocrine and paracrine mechanisms such as flow-induced vasodilation and humoral mediators. NO is produced by the human umbilical artery, where it may help to maintain vascular tension (Van de Voorde et al., 1987). In the present study, treatment with L-NMA, a competitive inhibitor of NO synthesis, increased vascular tension in artery sections with an intact endothelium. Furthermore, pretreatment with L-NMA significantly reduced the potentiating effect of melatonin on vascular tension. These findings suggest that the vasoconstrictive effect of melatonin may be at least partly mediated by inhibition or inactivation of basal NO release when endogenous NO synthesis is not suppressed, but when endogenous NO synthesis already has been suppressed by pretreatment with L-NMA, this vasoconstrictive effect of melatonin does not occur.

The specific mechanism underlying the vasoconstrictive effects of melatonin could involve an alteration of NO synthesis similar to that previously demonstrated in cerebral tissues. Melatonin acted as an NOS inhibitor at concentrations of $10^{-4}$ M or slightly higher in rat cerebellum (Pozo et al., 1994) and hypothalamic homogenate (Betthahi et al., 1996), and acted similarly at concentrations of $10^{-9}$ to $10^{-3}$ M in rat striatal homogenate (Leon et al., 1998). Inhibition of NO synthesis probably involves the binding of melatonin to calmodulin, thereby interfering with the binding of calmodulin to the NOS peptide that triggers synthesis (Ouyang and Vogel, 1998). In the present study, however, we found no significant effect of melatonin ($10^{-5}$ M) on calcium ionophore A 23187-induced relaxation, a phenomenon mediated by increased NO synthesis. This result suggests that melatonin at $10^{-5}$ M does not inhibit NO synthesis in the umbilical artery. The vasoconstrictive effect of melatonin must involve other mechanisms.

An alternative mechanism for the action of melatonin may involve NO scavenging. Noda et al. (1999) have demonstrated that at approximately equimolar or slightly higher concentrations ($10^{-4}$ to $5\times10^{-4}$ M), melatonin scavenges NO ($10^{-4}$ M) in vitro to a significant extent. As the half-life of NO is 3 to 30 s (Palmer et al., 1987; Beckman, 1991), the rapidity of the vasospastic effect of exogenous melatonin suggests that, under physiologic conditions, melatonin could act by scavenging NO. Normal concentrations of melatonin in human serum range from $6\times10^{-11}$ to $6\times10^{-10}$ M, depending on sampling time and subject age (Reiter, 1994, 1995). Concentrations of melatonin in serum from human umbilical cord ordinarily correspond to those in maternal serum (Okatani et al., 1998). Oral administration of melatonin (3 mg) to pregnant women near term produces a peak concentration in maternal serum of about $10^{-7}$ M after 1 to 2 h, with a corresponding concentration in umbilical cord serum (Okatani et al., 1998). The present study indicates that the vasospastic effect of melatonin is extremely weak and may be negligible at physiologic concentrations in umbilical cord plasma. Even at a pharmacologic concentration ($10^{-7}$ M), melatonin produced less than 10% of the umbilical artery vasospastic response seen with L-NMA in ex vivo preparations. As for the phasic constriction evoked by 5-HT, we previously found that pretreatment with L-NMA ($2\times10^{-4}$ M) significantly potentiated the 5-HT-induced constriction (Okatani et al., 1996). However, in the present study, pretreatment with melatonin ($10^{-5}$ M) did not affect the 5-HT-induced constriction. Thus, melatonin at $10^{-5}$ M

may not reduce bioavailable NO sufficiently to potentiate the 5-HT-induced constriction or to decrease the calcium ionophore A 23187-induced relaxation.

In conclusion, short-term exposure to melatonin potentiated vascular tension in human umbilical artery, perhaps by scavenging endothelially produced NO; inhibition of NO synthesis may not be involved. This vasoconstrictive effect of melatonin may be negligible at physiologic concentrations and is extremely low at pharmacologic concentrations below $10^{-7}$ M. The present results are important with regard to the clinical safety of treatment with melatonin. However, further clinical studies are needed to clarify the influence of exogenous melatonin on the feto-placental circulation.

## Acknowledgements

This work was supported by Research Grant 11671625 from the Ministry of Education of Japan.

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