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Melatonin and *N*-acetylserotonin inhibit leukocyte rolling and adhesion to rat microcirculation

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Abstract

The hormone melatonin produced by the pineal gland during the daily dark phase regulates a variety of biological processes in mammals. The aim of this study was to determine the effect of melatonin and its precursor *N*-acetylserotonin on the microcirculation during acute inflammation. Arteriolar diameter, blood flow rate, leukocyte rolling and adhesion were measured in the rat microcirculation in situ by intravital microscopy. Melatonin alone or together with noradrenaline did not affect the arteriolar diameter or blood flow rate. Melatonin inhibited both leukocyte rolling and leukotriene B_4 induced adhesion while its precursor *N*-acetylserotonin inhibits only leukocyte adhesion. The rank order of potency of agonists and antagonist receptor selective ligands suggested that the activation of MT_2 and MT_3 melatonin binding sites receptors modulate leukocyte rolling and adhesion, respectively. The effect of melatonin and *N*-acetylserotonin herein described were observed with concentrations in the range of the nocturnal surge, providing the first evidence for a possible physiological role of these hormones in acute inflammation.

Keywords: Inflammation; Microcirculation; Melatonin; Melatonin receptor; *N*-acetylserotonin

1. Introduction

Many biological processes follow diurnal rhythms related to the light–dark cycle. The mice paw thickness during chronic granulomatous inflammation shows a 24-h rhythm with minimal responses during the daily dark period (Lopes et al., 1997). Recently, we demonstrated that this rhythmic response is regulated by melatonin (Lopes et al., 2001), as the diurnal rhythm of inflammation is abolished by pinealectomy and restored by nocturnal replacement of melatonin to pinealectomized animals. Diurnal rhythms are also described in the acute inflammatory responses. Paw edema (Loubaris et al., 1982) and polymorphonuclear leukocytes migration induced by carrageenin (Garrelly et al., 1991) or Bacillus Calmette-Guerin (BCG) (Bureau et al., 1986) varies according to the hour of stimulus administration. Stimulus applied during the dark phase induced a lower inflammatory response than when applied during the light phase. A possible relationship between such rhythms and the nocturnal surge of melatonin hormone has not being described.

The protective effects of melatonin observed in local inflammation (Cuzzocrea et al., 1997; Costantino et al., 1998), experimental endotoxic (Maestroni, 1996) or nonseptic shock (Cuzzocrea et al., 1998), and ischemia–reperfusion injury in hamster cheek pouch (Bertuglia et al., 1996) were attributed to the putative antioxidant and radical scavenger action of melatonin. The physiological role of endogenous melatonin in these described models has been questioned, as the doses of exogenous melatonin required for inhibition of these responses was higher than the nocturnal maximal serum concentration. However, our recent data demonstrating that melatonin mediates the diurnal rhythm of chronic granulomatous inflammation (Lopes et al., 2001) suggests that doses of melatonin lower than those described as scavengers could modulate inflammatory responses. The effects of melatonin at low doses could be better explained by an action at membrane receptors.

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In mammals, melatonin activates at least three distinct high-affinity receptors, the MT_1, MT_2 and MT_3. The melatonin MT_1 and MT_2 membrane-bound receptors show 60% homology at the amino acid level and are encoded by separate gene. These two melatonin receptors can be distinguished pharmacologically both in vivo and in vitro using the selective MT_2 melatonin ligand 4-phenyl-2-propionamidotetralin (4P-PDOT) (Dubocovich, 1995; Dubocovich et al., 1997, 1998; Dubocovich and Massana, 1998). Acute inhibition of neuronal firing in the mouse suprachiasmatic nucleus (Liu et al., 1997) and arterial vasoconstriction in the rat (Krause et al., 1995; Doolen et al., 1998; Bucher et al., 1999; Ting et al., 1997, 1999) appear to be mediated through activation of the MT_1 melatonin receptor. By contrast, activation of the MT_2 melatonin receptor by melatonin inhibits dopamine release in retina (Dubocovich et al., 1997) and phase advances circadian rhythms of wheel running activity in the C3H/HeN mouse (Dubocovich et al., 1998).

The putative MT_3 melatonin receptor binds to 2-[^{125}I]-iodomelatonin and to the MT_3 selective radioligand 2-[^{125}I]-5-methoxycarbonylamino-*N*-acetyl-tryptamine (2-[^{125}I]-5-MCA-NAT) with nanomolar affinity and shows a pharmacological profile (2-iodomelatonin > prazosin > *N*-acetylserotonin ≥ melatonin) distinct from that of the high affinity sites (MT_1 and MT_2) where melatonin has significantly higher affinity than *N*-acetylserotonin (Dubocovich, 1988, 1995; Pickering and Niles, 1992; Molinari et al., 1996; Le Gouic et al., 1997). Recently, the MT_3 binding sites were suggested to be the enzyme quinone reductase 2 (Nosjean et al., 2000, 2001), which is a soluble flavoprotein that catalyzes the oxidation of reduced ribosyl nicotinamide by vitamin K_3. A protein purified by affinity chromatography using 2-[^{125}I]-5-MCA-NAT and pharmacologically characterized from Syrian hamster kidney was found to be homologous of the human quinone reductase 2. The physiological importance of this enzyme is unknown. Therefore, it is not known if the MT_3 is a classical membrane receptor or is an intracellular binding site, but it is accepted that it has a distinct pharmacological profile, being the only with high affinity to *N*-acetylserotonin when compared to melatonin and the only one responsive to 5-MCA-NAT.

In this work we intend to define the effects of physiological concentrations of melatonin on the first steps of acute inflammation. Furthermore, we show the pharmacologically profile of the receptors involved.

Initial inflammatory responses involve three major changes in the microcirculation. First, a transient vasoconstriction of arterioles followed by a vasodilation leads to an increase in local blood flow. Then, an increase in vascular permeability causes a slowing of the circulation. These first two events allow leukocytes, mainly neutrophils, that usually travel at the middle of the vessels, to marginate entering in contact with endothelium and initiating leukocyte recruitment.

Vascular reactivity is affected by melatonin. Melatonin enhances vascular tone in both peripheral and cerebral arteries (Ting et al., 1997; Geary et al., 1995) and potentiates neurogenic induced contraction in rat caudal artery (Krause et al., 1995; Geary et al., 1998; Lew and Flanders, 1999). Therefore, a potential mechanism by which melatonin may inhibit inflammatory responses is by decreasing blood flow due to arteriolar contraction.

Another possibility is that melatonin could modulate acute inflammatory responses by interfering with leukocyte recruitment. Migration of leukocytes to the adjacent tissue shows diurnal variation (Garrelly et al., 1991; Bureau et al., 1986). Migration of leukocytes from the vessels to the tissue occurs mainly at postcapillary venules as a result of interactions between leukocytes and endothelium (Granger and Kubes, 1994). As leukocytes marginate, they initiate a process called rolling, which is described as a low-affinity adhesive interaction with the endothelium whereby the force of blood flow acts on the leukocyte to induce a rotational motion. A class of adhesion molecules named selectin (Granger and Kubes, 1994) mediates leukocyte rolling. A stronger interaction of leukocytes to endothelial cells that precede transmigration is called adhesion, which is mediated by another class of adhesion molecules (integrin and immunoglobulin) (Granger and Kubes, 1994). Integrins are constitutively expressed in leukocytes in a low affinity state. changing toward a high affinity state in response to mediators such as leukotriene B_4 (Dahlen et al., 1981).

The aim of this study was to investigate if melatonin and its precursor *N*-acetylserotonin could play a direct role on arteriolar contraction, blood flow, leukocyte rolling and adhesion in rat microcirculation. We demonstrated that activation of melatonin receptors by melatonin inhibited both leukocyte rolling and adhesion, and by *N*-acetylserotonin inhibited only leukocyte adhesion. The effects herein described were obtained with doses of melatonin and *N*-acetylserotonin that are in the range of the nocturnal surge, providing the first evidence of a possible physiological role of these hormones in acute inflammation.

2. Methods

2.1. Melatonin receptor nomenclature and classification

Here, we use the official nomenclature for melatonin receptors approved by the Nomenclature Committee of the International Union of Pharmacology (IUPHAR) (Dubocovich et al., 1998, 2000). The designation "mt_1" and "mt_2" corresponds to that of the recombinant melatonin receptors previously known as Mel_{1a} (Reppert et al., 1994) and Mel_{1b} (Reppert et al., 1995, 1996), respectively. These receptors should be referred in upper case, i.e., MT_1 and MT_2, to reflect the pharmacological characterization

of their function in native tissues (Doolen et al., 1998; Dubocovich et al., 1997; Dubocovich and Massana, 1998; Ting et al., 1999). MT_3 refers to the pharmacologically defined melatonin receptor, previously referred to as ML2 (Dubocovich, 1995). Recently, a hamster kidney binding site that was identified as the quinone reductase 2 enzyme (Nosjean et al., 2000).

2.2. Animals

Male Wistar rats (200–300 g) were maintained in a 12/12-h light–dark cycle and allowed a standard diet and water ad libitum. All experiments were done at the light phase of the cycle.

2.3. Direct vital microscopy of the microcirculation

Rats were anesthetized with sodium pentobarbital (75 mg/kg, i.p.). The internal spermatic fascia of the scrotal chamber wall was exposed for microscopic examination in situ (Fortes et al., 1991; Farsky et al., 1995). The interna fascia was exposed by a longitudinal incision throughout the skin and dartos muscle in the midline over the ventral aspect of the scrotum and opening of the cremaster muscle. The animals were maintained on the top of a thermostatically kept board (37 °C). The tissue was trans-illuminated while placed on a transparent platform. The preparation was kept moist and warmed by irrigating the tissue with warmed (37 °C) Ringer–Locke's solution (pH 7.4) containing 1% gelatin. The composition of the solution was (mM): NaCl, 154.0, KCl, 5.6, $CaCl_2$, 2.0, $NaHCO_3$, 6.0 and glucose, 5.0. The exposed tissue was constantly humidified with the physiological solution. Images of the microcirculation were captured through a color video camera (TK-C600, JVC) incorporated into a triocular microscope (Axioskop, Carl-Zeiss) and were simultaneously visualized in a TV monitor and in a computer monitor. Images obtained on the TV monitor were recorded on a video and digitilized images in the computer were analyzed employing image analyzer software (KS300, Kontron). The images were obtained using a $\times 40/0.65$ longitudinal distance objective/numeric aperture and $\times 1.0$ or 1.6 optovar. Vessels selected for study were defined according to their branch order location within the microvascular network (Bohlen and Gore, 1977). The vessels correspond to post-capillary venules and small arterioles, and their diameters, measured by the image analyzer software, ranged from 15 to 25 μm. All drugs were topically administered to the microvascular network in a standard volume of 10 μl sterile saline.

Arteriolar diameter and blood flow rates were measured at varying intervals after melatonin (10 nM), noradrenaline (1 μM) or melatonin plus noradrenaline administration. The noradrenaline concentration was the minimal effective, which allows the observation of a possible melatonin potentiating effect. Blood flow rates were measured by using an Optical Doppler Velocimeter incorporated into the microscope. Arteriolar diameters of digitized images collected at different intervals were measured using the image analyzer software.

Leukocytes moving in the periphery of the axial stream of a selected post-capillary venule, in contact with the endothelium, were considered to be rollers (Dahlen et al., 1981). These leukocytes moved sufficiently slowly to be individually visible and were counted as they rolled past a selected point on one side of the vessel. The trauma caused by the surgical manipulation is sufficient to provide the leukocyte rolling observed. Leukocyte rolling values measured during 10 min before melatonin (0.1 pM–1 μM), *N*-acetylserotonin (1–10 μM), 4P-PDOT (0.1 nM–0.1 μM), luzindole (10 μM) or vehicle administration were used as basal value. The competitive melatonin receptor antagonist luzindole was added 10 min before melatonin. The number of rolling cells during an interval of 10 min, were counted after 10 min of drug administration.

Leukocyte adhesion was measured in a selected area of a post-capillary venule. The number of sticking leukocytes counted 10 min after leukotriene B_4 (0.3 μM) administration was used as basal value. Melatonin (0.1–100 nM), *N*-acetylserotonin (1 pM–10 nM), 5-MCA-NAT (10 pM–1 nM) or luzindole (10–300 μM) were administered 10 min after leukotriene B_4 administration. Luzindole was added together with leukotriene B_4 10 min before melatonin or *N*-acetylserotonin administration. The number of sticking leukocytes were counted after 20 min of drug administration. All experiments were done at the light phase of the cycle.

Table 1
Melatonin (MLT, 10 nM) effect on arteriolar diameter and blood flow rates with and without vasoconstriction induced by noradrenaline (NOR, 1 μM)

	Vehicle	MLT	NOR	NOR + MLT
Arteriolar diameter	105.27 ± 2.80 $n = 4$	98.01 ± 2.59 $n = 4$	90.89[a] ± 1.98 $n = 4$	94.32[a] ± 2.55 $n = 7$
Arteriolar blood flow rate	102.52 ± 3.60 $n = 4$	99.73 ± 1.26 $n = 8$	103.49 ± 5.80 $n = 4$	95.78 ± 5.79 $n = 6$
Venular blood flow rate		100.29 ± 3.84 $n = 4$		

Data are presented as percentage of the basal value and are shown as means ± S.E.M. of the number of animals indicated.
[a] Significantly different from vehicle ($P < 0.05$).

2.3.1. Data analysis

Data are presented as percentage of the basal values. This normalization is necessary because of the high individual variability observed. Data were analyzed by Student *t* test or analysis of variance (ANOVA), when one or more than one means were compared, respectively. In the last case, the difference among the means was tested by Newman–Keuls test (GraphPad Prism software). The number of experiments denote the number of independent animals tested, as only one concentration could be tested in each animal. EC_{50} values were obtained from de nonlinear adjusted curves (GraphPad Prism software).

2.4. Drugs

Melatonin, *N*-acetylserotonin, noradrenaline and luzindole were obtained from Sigma (St. Louis, MO). Leukotriene B_4 was purchased from ICN Pharmaceutical (Irvine, CA). 4P-PDOT and 5-MCA-NAT were obtained from Tocris (Ballwin, MO). Melatonin, *N*-acetylserotonin, luzindole, 4P-PDOT and 5-MCA-NAT were dissolved initially to 10 mM in 10% ethanol, while luzindole and 4P-PDOT were dissolved in 100% of ethanol. All drugs were further diluted in sterile saline to the desired final concentration. In control experiments, vehicle was added with the same ethanol amount used in the dilution of the highest drug concentration applied. Noradrenaline was diluted in sterile saline plus ascorbic acid (50 mg/l).

3. Results

3.1. Arteriolar diameter and blood flow rate

Noradrenaline (1 μM) reduced arteriolar diameter but did not affect blood flow. Melatonin (10 nM) alone or added together with noradrenaline did not change arteriolar diameter or venular blood flow rate (Table 1).

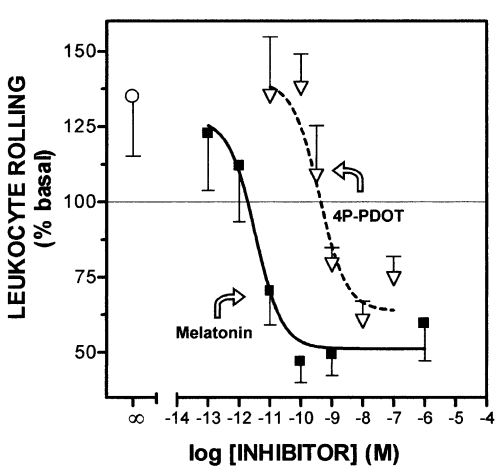

Fig. 1. Melatonin and 4P-PDOT inhibited leukocyte rolling. Leukocyte rolling was determined 10 min after topical application of melatonin, 4P-PDOT or vehicle (open circle). −log EC_{50} for melatonin and 4P-PDOT are 11.47 ± 0.37 and 9.39 ± 0.41, respectively. Data are shown as mean ± S.E.M. of eight animals in vehicle, and 3–4 animals per concentration of agonists.

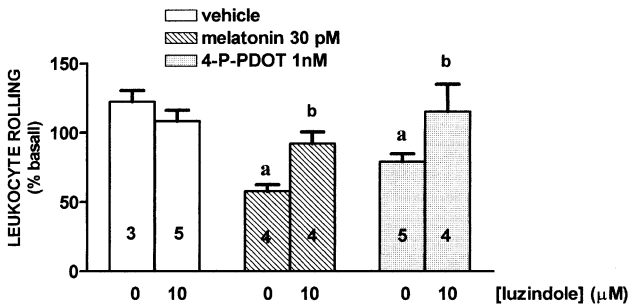

Fig. 2. Luzindole blocks melatonin and 4P-PDOT inhibition of leukocyte rolling. Luzindole (10 μM) was administered 10 min before vehicle, melatonin (30 pM) or 4P-PDOT (1 nM). Leukocyte rolling was measured 10 min after topical application of melatonin, 4P-PDOT, vehicle or luzindole. The basal values were measured 10 min before drug application. Data shown represent mean ± S.E.M of the number animals indicated on graph. [a] $P < 0.001$ when compared to vehicle. [b] $P < 0.01$ when compared to melatonin or 4P-PDOT in the absence of luzindole.

3.2. Leukocyte rolling

Leukocyte rolling in a selected postcapillary venule measured 10 min before drug administration was taken as basal value. Melatonin inhibited leukocyte rolling in a concentration dependent manner ($-\log EC_{50} = 11.47$, ± 0.37, $n = 3$ to 4 animals per group) (Fig. 1). The selective melatonin MT_2 receptor analogue 4P-PDOT mimicked melatonin effect on leukocyte rolling ($-\log EC_{50} = 9.39 \pm 0.41$, $n = 3$ to 4 animals per group). The melatonin precursor, *N*-acetylserotonin (1 and 10 μM), the

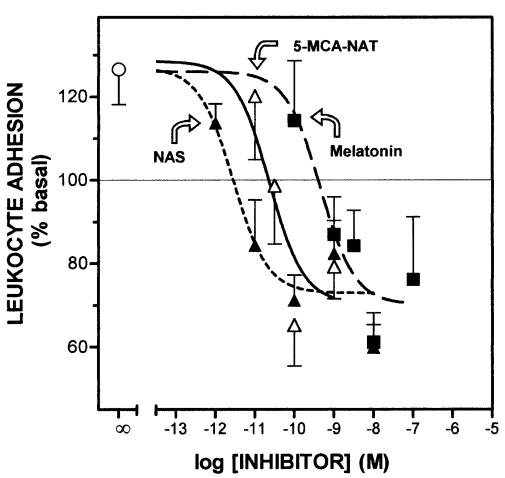

Fig. 3. Melatonin, *N*-acetylserotonin (NAS) and 5-MCA-NAT inhibited leukocyte adhesion. Adhesion was stimulated by 0.3 μM of leukotriene B_4. Melatonin ($-\log EC_{50} = 9.33 \pm 0.41$), *N*-acetylserotonin ($-\log EC_{50} = 11.55 \pm 0.38$), 5-MCA-NAT ($-\log EC_{50} = 10.63 \pm 0.37$) or vehicle (open circle) were topically administered 10 min after leukotriene B_4 stimulation. Only one concentration of drug or vehicle was administered per rat. Data shown are means ± S.E.M. of seven animals in vehicle, and 3–4 animals per concentration of agonist.

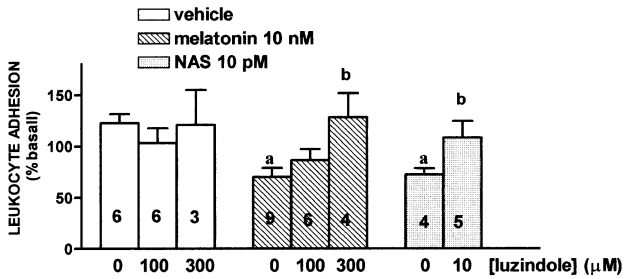

Fig. 4. Luzindole blocks melatonin and *N*-acetylserotonin effects on leukocyte adhesion. Luzindole (10, 100 or 300 μM) was administered with leukotriene B_4 10 min before melatonin (10 nM) or *N*-acetylserotonin (NAS, 10 pM) application. Data are shown as mean ± S.E.M. of the number of animals indicated on graph. [a] $P < 0.05$ when compared with vehicle. [b] $P < 0.05$ when compared to melatonin or *N*-acetylserotonin in the absence of luziondole.

selective MT_3 analogue 5-MCA-NAT (1 nM) (not shown) and the non-selective and competitive melatonin receptor antagonist luzindole (10 μM) (Fig. 2) did not affect leukocyte rolling when added alone. However, luzindole (10 μM) blocked the inhibition of leukocyte rolling induced by melatonin (30 pM) and 4P-PDOT (1 nM) (Fig. 2).

3.3. Leukocyte adhesion

Leukocyte adhesion measured 10 min after stimulation with leukotriene B_4 (0.3 μM) was taken as basal value. Leukocyte adhesion was inhibited in a concentration dependent manner by either melatonin ($-\log EC_{50} = 9.33 \pm 0.41$, $n = 3$ to 7 animals per group), *N*-acetylserotonin ($-\log EC_{50} = 11.55 \pm 0.38$, $n = 3$ to 7 animals per group) or 5-MCA-NAT ($-\log EC_{50} = 10.63 \pm 0.37$, $n = 3$ to 7 animals per group) (Fig. 3). The analogues 4P-PDOT (1 nM) (data not shown) and luzindole (10 μM) (Fig. 4) have no effect on leukocyte adhesion per se. Melatonin and *N*-acetylserotonin inhibition of leukocyte adhesion were blocked by previous administration of the competitive melatonin receptor antagonist luzindole (300 and 10 μM, respectively) (Fig. 4).

4. Discussion

In this study, melatonin and its precursor, *N*-acetylserotonin, inhibit acute inflammatory responses in the microcirculation by activation of pharmacologically distinct melatonin receptors. The inhibition of leukocyte rolling and adhesion is not mediated through changes in arteriolar contraction or blood flow rate in the rat microvasculature. We demonstrated that activation of a high affinity melatonin receptor (MT_2) mediates inhibition of leukocyte rolling, while the MT_3 binding site melatonin receptor modulates leukocyte adhesion. The doses of melatonin and *N*-acetylserotonin affecting leukocyte rolling and adhesion are well in the range of the nocturnal surge of these hormones, providing the first evidence for a possible physiological role in acute inflammation.

The inflammatory response depends upon changes into the microcirculation, which culminate in the transmigration of cells from the blood stream to the adjacent tissue. Leukocyte interactions with endothelial cells is a two-step process (rolling and adhesion), which is dependent on the balance between the adhesive forces of adhesion molecules against the hydrodynamic forces (shear rate, shear stress) generated by the movement of blood within the microcirculation. The prevailing wall shear rate determines the levels of leukocyte rolling and firm adhesion, and it dictates the area between rolling leukocytes and the endothelial cell surface (Bienvenu and Granger, 1993). Melatonin can alter vascular reactivity in certain arteries (Ting et al., 1997), but not the in microcirculation. Melatonin is unable to affect arteriolar diameter, blood flow rates, or to potentiate the responses induced by noradrenaline. In summary, the effect of melatonin on acute inflammation reported here is not due to changes in arteriolar reactivity or hemodynamics.

Melatonin inhibits leukocyte rolling and adhesion to endothelial cells of the microvasculature by activation of distinct melatonin binding sites receptors. The potency of melatonin to inhibit leukocyte rolling ($EC_{50} = 3.4$ pM) was approximately 130-fold higher than to inhibit leukotriene B_4-induced leukocyte adhesion ($EC_{50} = 458.7$ pM). On the other hand, the precursor of melatonin, *N*-acetylserotonin ($EC_{50} = 2.8$ pM) was approximately 160 times more potent than melatonin to inhibit leukotriene B_4-induced leukocyte adhesion. Melatonin and 4P-PDOT effects on rolling and melatonin, 5-MCA-NAT and *N*-acetylserotonin effects on adhesion were blocked by the non-selective (MT_1, MT_2 and MT_3) melatonin receptor antagonist, luzindole (Dubocovich, 1988, 1995; Popova and Dubocovich, 1995). Protective effects of melatonin on acute inflammation were observed with high pharmacological concentrations of melatonin (Cuzzocrea et al., 1997, 1998; Costantino et al., 1998; Maestroni, 1996; Bertuglia et al., 1996). These effects of the hormone were attributed to its free radical scavenger and antioxidant properties. Our results suggest that the acute antiinflamatory effects of physiological concentrations of melatonin be mediated through activation of melatonin binding sites.

Melatonin-mediated inhibition of leukocyte rolling appears to result from activation of a high affinity melatonin receptor, probably the MT_2. Competition of 4P-PDOT for 2-[^{125}I]-iodomelatonin binding to recombinant melatonin MT_2 receptors stably expressed in cells lines of identical background showed over 1000 times higher affinity (Ki = 0.41) than for the MT_1 melatonin receptor (Dubocovich et al., 1998; Nonno et al., 1999). The melatonin analogue 4P-PDOT is a high affinity and selective antagonist of the MT_2 melatonin receptor (Dubocovich et al., 1997, 1998; Dubocovich and Massana 1998). However, in the present

study, this selective MT_2 melatonin receptor analogue 4P-PDOT acted as an agonist on leukocyte rolling, and showed no effect on leukotriene B_4-induced leukocyte adhesion. This agonistic effect is blocked by the previous administration of luzindole, indicating that, indeed, this effect is due to melantonin receptor activation. In cell lines expressing high density of recombinant MT_2 melatonin receptors, 4P-PDOT acted as partial agonist (Dubocovich and Massana, 1998; Nonno et al., 1999). Taken together, it is therefore likely that the inhibition of leukocyte rolling by 4P-PDOT is due to activation of melatonin MT_2 receptors in the microvasculature.

The MT_3 melatonin binding site appears to mediate inhibition of leukotriene B_4-induced leukocyte adhesion in microcirculation. Both *N*-acetylserotonin (EC_{50} = 2.8 pM) and the selective MT_3 agonist 5-MCA-NAT (EC_{50} = 23.4 pM) inhibited leukotriene B_4-induced leukocyte adhesion with a pharmacological order of potency expected for a MT_3 melatonin binding site (Dubocovich, 1995; Popova and Dubocovich, 1995). The high potency of *N*-acetylserotonin in inhibiting leukocyte adhesion and the effect of the selective MT_3 analogue 5-MCA-NAT suggests that this effect is mediated by the putative MT_3 receptor/or binding site. The selective putative antagonist prazosin was tested (data not shown), however, as this drug is an its α-adrenoceptor antagonist properties caused hemodynamic changes that impaired the effect of leukotriene B_4 to induce leukocyte adhesion and, therefore, the inhibitory effect of melatonin could not be tested. The MT_3 receptor was identified as the enzyme quinone reductase 2 (Nosjean et al., 2000). If this enzyme is present in the microcirculation one could expect that inhibition of the quinone reductase 2 would also inhibit leukocyte adhesion. However, the physiological importance of this enzyme is, at the moment, unknown. Therefore, it is not possible to correlate the quinone reductase 2 inhibition with the melatonin and *N*-acetylserotonin effects on leukocyte adhesion.

Melatonin and *N*-acetylserotonin-mediated inhibition of inhibited leukocyte rolling and adhesion occurs within the physiological range of concentrations. Therefore, endogenous *N*-acetylserotonin may play a role in modulating leukocyte adhesion to endothelial cells in acute inflammation as nocturnal plasma levels in rats may reach a concentration of 10 pM (Pang et al., 1980). The potency of melatonin in reducing leukocyte rolling and adhesion also suggests a physiological role for the endogenous hormone in inflamation. If this is the case, the lower inflammatory acute responses observed when the inflammatory stimulus is given during the dark phase (Loubaris et al., 1982; Garrelly et al., 1991; Bureau et al., 1986) could be due to melatonin and *N*-acetylserotonin inhibition of leukocyte migration.

In summary, while melatonin inhibits leukocyte rolling and adhesion, *N*-acetylserotonin reduces only leukocyte adhesion. The effect of both drugs was blocked by luzindole and mimicked by synthetic agonists, suggesting that these effects are mediated by melatonin membrane receptors. The difference in the potency between melatonin and NAS in the two processes suggests that each one is mediated by a different receptor (rolling-MT_2 receptor, adhesion-MT_3 binding site). The effective doses of melatonin and *N*-acetylserotonin strongly indicate that the endogenous hormones play a role in modulating acute inflammation.

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