ELSEVIER

European Journal of Pharmacology 395 (2000) 15–21

ejp

www.elsevier.nl/locate/ejphar

Melatonin reversal of lipopolysacharides-induced thermal and behavioral hyperalgesia in mice

Vasudeva Raghavendra [a], Javed N. Agrewala [b], Shrinivas K. Kulkarni [a,*]

[a] *Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India*
[b] *Institute of Microbial Technology, Sector-39 A, Chandigarh 160036, India*

Received 23 September 1999; received in revised form 25 February 2000; accepted 3 March 2000

Abstract

The perception of pain sensation (threshold), whether local or central, is altered by inflammatory processes. Anti-inflammatory drugs block this by raising the pain threshold and by reducing the inflammatory process. Melatonin is claimed to have anti-inflammatory activity in animal models of acute and chronic inflammation. However, it is not known whether melatonin can reverse the hyperalgesia that is secondary to the inflammation. The present study aimed to assess the modulatory effect of melatonin on lipopolysacharides-induced alteration of pain perception in mice. Central perception of pain was assessed with the tail-flick and hot-plate methods and local hyperalgesia was assessed by noting the animal's reactions such as paw licking and rearing after the intraplantar injection of lipopolysacharides (5 μg/paw). Local administration (intraplantar) of lipopolysacharides induced hyperalgesia when measured by both central effects and behavioral reactions. Melatonin (5 and 10 mg/kg), like dexamethasone (0.5 mg/kg), given 30 min prior to, and 4 and 8 h after lipopolysacharides (5 μg/paw) challenge attenuated central and behavioural hyperalgesia. The attenuation of lipopolysacharides-induced hyperalgesia by melatonin was not reversed by naltrexone (4 mg/kg). In vitro studies showed that melatonin, in concentrations ranging from 100 to 1000 nM, suppressed tumor necrosis factor-α (TNF-α) without affecting the nitric oxide (NO) release in lipopolysacharides-activated murine peritoneal macrophages. Taken together, the present results demonstrated that melatonin reverses lipopolysacharides-induced hyperalgesia.

Keywords: Lipopolysacharide; Melatonin; Dexamethasone; Naltrexone; TNF-α (tumor neocrisis factor-α); Nitric oxide (NO); Hyperalgesia, central/peripheral

1. Introduction

Illness-induced hyperalgesia is one of the most common aspects of pain-related inflammation and the therapeutic approach to this pain should aim at interfering with various mediators of the inflammatory reactions, including neuropeptides, eicosanoids and cytokines (Dray and Bevan, 1993). The intraplantar (i.pl.) injection of lipopolysacharides, an endotoxin, induces central sensitization thereby reducing the threshold for nociceptive stimulus in the tail-flick and hot-plate tests (Kanaan et al., 1996). Endotoxin-induced hyperalgesia, which has a short duration, is completely reversible and causes minimum distress to the animal as compared to the effect of an adjuvant, carragenin or formalin (Kanaan et al., 1996). Besides, the inflammation and hyperalgesia induced by lipopolysacharides involve the release of cytokines during bacterial infections (Rietschell et al., 1994). This may allow the study of the action of the various classes of anti-inflammatory and analgesic drugs (Kanaan et al., 1997).

The role of melatonin as an immunomodulator is well established (Liebmann et al., 1997). A recent report showed that melatonin exerts protective effects in septic and hemorrhagic shock (Sacco et al., 1998), reverses chronic and acute inflammation (Missbach et al., 1996; Cuzzocrea et al., 1997; Costantino et al., 1998) and endotoxin-induced sickness behaviors like adepsia and hyperthermia (Nava et al., 1997; Raghavendra et al., 1999). However, so far no report has addressed the modulation of inflammatory hyperalgesia by melatonin. Because of this, the present study was carried out to test the effect of melatonin on lipopolysacharides-induced inflammatory hyperalgesia.

* Corresponding author. Telefax: +91-172-541142.
E-mail address: skpu@yahoo.com (S.K. Kulkarni).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00196-5

The effect of melatonin on lipopolysacharides-induced hyperalgesia was evaluated using the tail-flick and hot-plate methods, and pain-related behavior in mice was also observed. The effect of melatonin was compared with that of dexamethasone, a known inhibitor of lipopolysacharides-induced hyperalgesia and behaviors (Kanaan et al., 1997). To study the effect of the interaction between melatonin and lipopolysacharides on the immune system, in vitro experiments were performed using thioglycollate elicited macrophages.

2. Materials and methods

2.1. Animals

Inbred male Balb/c mice (25–30 g), which were obtained from the Institute Animal House, were used. The animals were housed under optimal conditions with food and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee and experiments were performed with strict adherence to the ethical guidelines for the study of pain in conscious animals (Zimmerman, 1983).

2.2. Drugs and reagents

Escherichia coli lipopolysacharides (serotype 0111:B4) and naltrexone were from Sigma (St. Louis, MO, USA). Melatonin was kindly provided by Morepen Laboratory (Parwano, India) and dexamethasone was obtained from Unichem Lab. (Bombay, India). RPMI 1640 medium, fetal calf serum, penicillin, streptomycin and thioglycollate broth were purchased from Gibco (Paisley, USA). *N*-(1-naphtyl)-ethylene diamine hydrochloride and sulfanilamide were obtained from Loba Chemie (Bombay, India). Recombinant tumor necrosis factor-α (TNF-α) was obtained from Genzyme (Cambridge, UK).

2.3. Behavioral assessment of pain

The animals were placed in a Plexiglass box ($45 \times 30 \times 30$ cm) and observed for licking and rearing behaviors for a period of 5 min. Increased licking of an injected paw shows discomfort caused by localized pain due to inflammogens, and a decrease in rearing behavior so to avoid pain sensation due to standing on hind legs. A score of 0, 1, 2 or 3 was given depending on the severity of these behaviors. The general state of reaction to pain in each group of animals was assessed from the mean scores of individual behaviors and was plotted on a scale ranging from 0 to 3. Behavioral scores were analyzed by non-parametric Kruskal–Wallis analysis of variance (ANOVA) followed by post-hoc comparison by Mann–Whitney's *U*-test.

2.4. Assessment of thermal hyperalgesia

2.4.1. Hot-plate test

The hyperalgesic response on the hot plate is considered to result from a combination of central and peripheral mechanisms (Kanaan et al., 1996). In this test, animals were individually placed on a hot plate (Eddy's Hot Plate) with the temperature adjusted to 50°C. The latency to the first sign of paw licking or jump response to avoid heat pain was taken as an index of pain threshold.

2.4.2. Tail-flick test

The hyperalgesic response in the tail-withdrawal test is generally attributed to central mechanisms (Kanaan et al., 1996). Tail withdrawal (flicking response) from the radiant heat source (Techno, Ambala, India) is taken as the endpoint. The intensity of the radiant heat was adjusted so that the baseline tail withdrawal of mice was within 4–5 s (Raghavendra and Kulkarni, 1999).

The nociceptive threshold, i.e., the latency (in s) to respond to noxious stimuli in both hot-plate and tail-flick tests was expressed as percentage of the baseline response as calculated from: $100 \times$ nociceptive threshold after lipopolysacharides/nociceptive threshold before lipopolysacharides. The percentage of baseline response obtained in saline (instead of lipopolysacharides)-injected animals was taken as control. The hyperalgesic response was analyzed by ANOVA followed by Dunnett's *t*-test.

2.5. Experimental protocol

Hyperalgesia in mice was induced by 5 μg of lipopolysacharides in 50 μl of saline injected into the left hind paws of mice (i.pl.). Melatonin (5 and 10 mg/kg dissolved in 1% ethanol) and dexamethasone (0.5 mg/kg) were administered subcutaneously (s.c.) 30 min before and 4 and 8 h after lipopolysacharides injection to maintain the appropriate level of the drugs. In separate groups of animals the opioid receptor antagonist, naltrexone (4 mg/kg), was co-administered with melatonin (5 mg/kg) to assess the possible role of μ opioid receptors in melatonin action. Behavioral (rearing and licking) responses and the central hyperalgesic effect in tail-flick and hot-plate tests were recorded at 6 and 10 h after lipopolysacharides injection.

2.6. Preparation of thioglycollate-elicited peritoneal macrophages

Male Balb/C mice (weighing 25–30 g) were inoculated intraperitoneally with 1 ml of a sterile 3% thioglycollate broth solution in a phosphate-buffered saline. The animals were killed 3 days later by decapitation. Peritoneal cells were collected by washing the peritoneal cavity several times with sterile, ice-cold phosphate-buffered saline. The lavages were centrifuged for 5 min (1000 rpm), the supernatants were removed, and the pellets were resus-

pended in 5 ml of the phosphate-buffered saline, pooled once again and centrifuged. After removal of the supernatants, the cells were resuspended in RPMI 1640 with a 10% heat-inactivated fetal calf serum, penicillin and streptomycin (each at a concentration of 100 U/ml) and 2.2% (W/V) $NaHCO_3$. The proportion of macrophages was enriched by the adherence of about 1.25×10^6 cells/ml for 2 h on plastic Petri dishes at 37°C under 7% CO_2. The non-adherent cells were removed by suction and washing out with pre-warmed RPMI 1640. The adherent cells (macrophages) were collected by gentle scraping. The purity of macrophages obtained routinely exceeded 95% as estimated by FACScan.

2.7. Incubation and stimulation conditions of peritoneal macrophages

Macrophages (1×10^5 cells/well) were incubated with or without melatonin (1000 nM) in the absence of lipopolysacharides (control) or in its presence (stimulating conditions) in complete medium (RPMI 1640 + 10% fetal calf serum) at 37°C under 7% CO_2. For estimation of TNF α and nitric oxide (NO), culture supernatants were collected 8 and 16 h, respectively, after lipopolysacharides challenge.

2.8. Determination of nitrite

Nitrite is a stable end-product of NO in vitro systems. Accumulation of nitrites was measured in cell-free supernatants by a spectrophotometric assay based on the Griess reaction (Di Rosa et al., 1990). Briefly, after 16 h of incubation, the medium from each well was mixed with an equal volume of Griess reagent (1% sulphanilamide/0.1% naphthylethylenediamine dihydrochloride/2.5% H_3PO_4), and incubated at room temperature for 10 min to yield a chromophore. Absorbance was read at 543 nm using a microplate reader (Eurogenetics, Torino, Italy). The nitrite concentration was calculated from a standard curve and expressed as μM/ml in three independent experiments. In all of the experiments, the nitrite content of wells containing medium without cells was measured and subtracted.

2.9. Bioassay of TNF-α

TNF-α was assayed by its ability to inhibit the proliferation of WEHI-164 cells (Lang et al., 1995). Briefly, WEHI-164 cells were cultured in 96-well flat-bottomed plates at a density of 1×10^5 cells/ml with different concentrations of culture supernatants from control and melatonin-treated wells. The cells were incubated for 12 h at 37°C pulsed with 0.5 μCi/well of [^{3}H]thymidine and harvested 8 h later. Incorporated radioactivity was measured using liquid scintillation spectrometry. Recombinant TNF-α (Genzyme) served as a standard to extrapolate the cytokine activity in terms of pg/ml.

Values from in vitro experiments are expressed as means ± S.D. The data were analyzed by ANOVA followed by Dunnett's *t*-test.

3. Results

3.1. Effect of melatonin on lipopolysacharides-induced hyperalgesia

The i.pl. injection of lipopolysacharides (5 μg) led to a significant decrease in rearing behaviour and increase in licking of the injected paw as compared to those of saline-treated animals [at 6 and 10 h after lipopolysacharides, $H(4) = 21.9$ and 23.3, $P < 0.05$, respectively, for rearing; $H(4) = 20.9$ and 27.5, $P < 0.05$ for licking]. Injection of melatonin (5 and 10 mg/kg) or dexamethasone (0.5 mg/kg) significantly attenuated the behavioral response to lipopolysacharides (Fig. 1). The administration of naltrexone (4 mg/kg) did not modify the behaviours (rearing and licking) induced by local injection of lipopolysacharides (data not shown). Naltrexone also failed to reverse the attenuating effect of melatonin (5 mg/kg) on the lipopolysacharides-induced behavioral response (Table 1).

The i.pl. injection of lipopolysacharides (5 μg) to mice produced a significant decrease in nociceptive threshold in

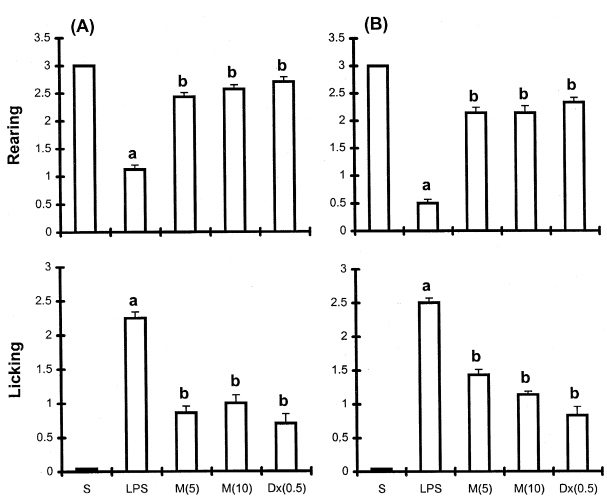

Fig. 1. Effect of melatonin (5 and 10 mg/kg, s.c.) and dexamethasone (0.5 mg/kg, s.c.) on rearing (upper panel) and licking (lower panel) scores in mice when tested 6 h (A) and 10 h (B) after intraplantar injection of lipopolysacharides (5 μg). Values are the average scores for each behaviour observed for a period of 5 min. [a] $P < 0.05$ as compared to saline and [b] $P < 0.05$ compared to lipopolysacharides alone-treated group (Kruscal–Wallis ANOVA followed by post-hoc comparison by Mann–Whitney's *U*-test).

Table 1
Failure of naltrexone (4 mg/kg, s.c.) to reverse melatonin the (5 mg/kg, s.c.) attenuation of lipopolysacharides (LPS; 5 μg, intraplantar)-induced hyperalgesia
Each response was recorded 10 h after lipopolysacharides (LPS; 5 μg) injection. Values are expressed as means ± S.E.M.

Treatment	*n*	Rearing	Licking	Tail-flick latency (% of baseline)	Hot plate latency (% of baseline)
Saline	8	3 ± 0	0 ± 0	100.4 ± 1.3	100.3 ± 1.1
LPS	8	0.5 ± 0.07[a]	2.5 ± 0.07[a]	69.4 ± 2.5[a]	65.9 ± 2.0[a]
Melatonin + LPS	7	2.1 ± 1.43[b]	1.4 ± 0.08[b]	79.5 ± 2.0[b]	80.8 ± 2.5[b]
Melatonin + LPS + naltrexone	6	2.2 ± 0.13[b]	1.3 ± 0.09[b]	82.9 ± 2.0[b]	81.4 ± 2.7[b]

[a] $P < 0.05$ as compared to saline.
[b] $P < 0.05$ compared to lipopolysacharides alone-treated group.

the hot-plate and tail-flick tests at 6 and 10 h after endotoxin injection. At the doses studied, melatonin (5 and 10 mg/kg) or dexamethasone (0.5 mg/kg) did not show an analgesic effect but significantly inhibited lipopolysacharides-induced hyperalgesia in the tail-flick and hot-plate tests at both time intervals [at 6 and 10 h after lipopolysacharides, $F(4,31) = 20.1$ and 31.9, $P < 0.05$, respectively, for tail-flick test; $F(4,31) = 20.8$ and 48.1, $P < 0.05$ for hot-plate test] (Fig. 2). The reversal of lipopolysacharides-induced hyperalgesia by both doses of melatonin (5 and 10 mg/kg) was of similar magnitude. Naltrexone (4 mg/kg) given alone or to lipopolysacharides-treated mice did not modulate the pain threshold in either hot-plate or tail-flick test (data not shown), and also failed to reverse the attenuating effect of melatonin (5 mg/kg) on lipopolysacharides-induced hyperalgesia (Table 1).

3.2. Effect of melatonin on in vitro release of TNF-α and NO from lipopolysacharides-activated macrophages

As expected, lipopolysacharides (1 μg/ml) induced an increase in TNF-α and NO production from thioglycollate-elicited macrophages. Melatonin (1–1000 nM) alone had no effect on baseline TNF-α and NO release from non-stimulated macrophages. However, when added 30 min prior to lipopolysacharides challenge, it dose depen-

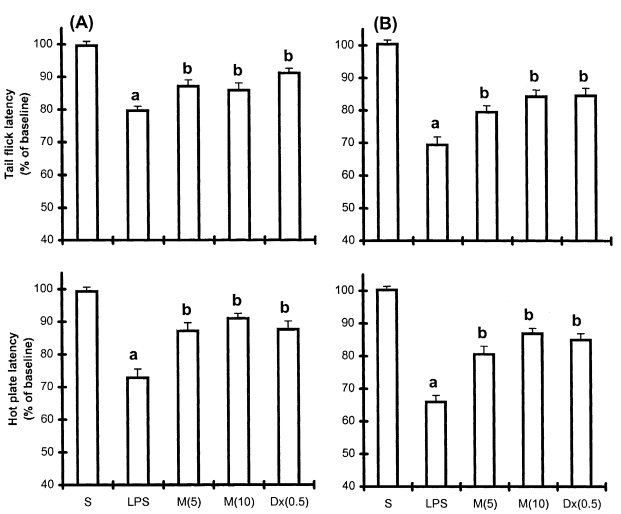

Fig. 2. Effect of melatonin (5 and 10 mg/kg, s.c.) and dexamethasone (0.5 mg/kg, s.c.) on tail-flick (upper panel) and hot-plate (lower panel) latencies in mice when tested 6 h (A) and 10 h (B) following the intraplantar injection of lipopolysacharides (5 μg). Values are means ± S.E.M. of % of baseline response. [a] $P < 0.05$ as compared to saline and [b] $P < 0.05$ compared to lipopolysacharides alone-treated group (ANOVA followed by Dunnett's *t*-test).

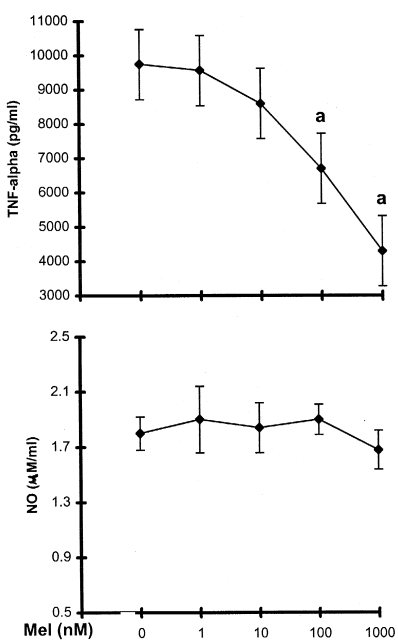

Fig. 3. In vitro effect of melatonin (1–1000 nM) on lipopolysacharide (1 μg/ml)-induced TNF-α (upper panel) and NO (lower panel) release from thioglycollate-elicited murine macrophages. Data expressed in means ± S.D. from triplicate samples. [a] $P < 0.05$ vs. lipopolysacharides alone (ANOVA followed by Dunnett's *t*-test).

dently suppressed TNF-α release without affecting NO production (Fig. 3).

4. Discussion

The results here presented show that melatonin inhibited the central hyperalgesia in response to thermal stimulation and suppressed the pain-related behaviors resulting from i.pl. injection of lipopolysacharides in mice. Although the precise site and mechanism of the anti-nociceptive effect of melatonin were not addressed in the present study, it would seem likely that melatonin reduces endotoxin-induced hyperalgesia as a consequence of inhibition of the immune regulatory effect of lipopolysacharides.

The drug administration schedule, i.e., injection of melatonin or dexamethasone before and after the induction of inflammation allowed their maintenance at a certain plasma level which may help to prevent the central hyperalgesia secondary to the peripheral hyperalgesia induced by local inflammation (Saade et al., 1993; Woolf, 1994). It was previously reported that morphine-class drugs were most effective for suppressing inflammogen-induced central and peripheral hyperalgesia (Hylden et al., 1991). Melatonin was known to modulate immune functions and to induce analgesia by interaction with endopioids, an effect which was antagonized by μ-receptor antagonists (Maestroni and Conti, 1991; Golombek et al., 1995). However, the dose of melatonin used in the present study was much lower than the analgesic dose, and also, naltrexone failed to block the melatonin reversal of lipopolysacharides hyperalgesia. This indicated that melatonin might interact directly with the immune system to attenuate the lipopolysacharides action. This observation gains support from the observation that melatonin binding sites are found on lymphocytes and macrophages (Pozo et al., 1997; Garcia-Perganeda et al., 1999).

The actions of pro-inflammatory cytokines due to peripheral administration of lipopolysacharides may also account for central nervous system (CNS) responses (Quan et al., 1999). Peripheral or intracerebroventricular (i.c.v.) injection of specific antagonists against these molecules is known to attenuate central effects of peripherally administered lipopolysacharides (Rothwell and Hopkins, 1995). Besides, specific antagonists of mediators that are released after pro-inflammatory cytokine activation (e.g., prostaglandins, kinins and NO, etc.) also differentially reverse central effects of lipopolysacharides (Rothwell and Hopkins, 1995; Raghavendra et al., 1999). It was previously reported that melatonin has a protective role in lipopolysacharides-induced septic shock and reverses adepsia and hyperthermia by suppressing pro-inflammatory cytokines, prostaglandins and NO production (Nava et al., 1997; Sacco et al., 1998; Raghavendra et al., 1999). In vitro experiments carried out during the present study showed that melatonin dose dependently suppressed the TNF-α release by lipopolysacharides-activated macrophages. Suppression of TNF-α could be one of the means by which melatonin attenuated the lipopolysacharides-induced hyperalgesia in mice.

Nuclear factor kappa B (NFκB) activation is supposed to be one of the principal mechanisms by which lipopolysacharides induces its cellular processes (Muller et al., 1993; Rietschell et al., 1994). The NFκB thus activated by lipopolysacharides induces many inflammatory genes that encode for pro-inflammatory cytokines, chemokines that selectively induce the inflammatory enzymes such as inducible NO synthase, cyclooxygenase-2, adhesion molecules and inflammatory receptors (e.g., interleukin-2 receptors) (Bauerle and Henkel, 1994). These released mediators contribute to the expression of inflammation and hyperalgesia. In in vitro studies on macrophage cell lines, melatonin was found to inhibit lipopolysacharides-induced activation of NFκB and thereby suppress the production of NO and 6-keto-prostalandin$_{2\alpha}$, principal mediators of inflammation and hyperalgesia (Gilad et al., 1998). Melatonin is also known to suppress TNF-α, phorbol ester and irradiation-induced NFκB activation (Mohan et al., 1995; Chuang et al., 1996). However, this pathway may not be the only one by which melatonin antagonizes lipopolysacharides action. In vitro tests performed during the present study showed that melatonin at a concentration as low as 100 nM was effective to reverse lipopolysacharides-induced TNF-α secretion without affecting the release of NO. This was found to be the case even at the highest concentration (i.e., 1000 nM) tested in the present study. Gilad et al. (1998) showed that the inhibitory action of melatonin on lipopolysacharides-induced NFκB activation occurred at a high micromolar concentration range (>10 μM). These observations showed that inhibition of lipopolysacharides-induced TNF-α release by melatonin may be due to its action on other regulatory sites by which lipopolysacharides exerts its action. Recently, Bulger et al. (1998) showed a transcriptional and functional enhancement of lipopolysacharides-induced TNF-α production despite inhibition of NFκB by dithiocarbamate in rabbit alveolar macrophages, thus indicating the existence of other pathways for lipopolysacharides-induced TNF-α production. Novogrodsky et al. (1994) reported that inhibitors of tyrosine kinases inhibit TNF-α production from murine macrophages after in vivo challenge with lipopolysacharides while they inhibit phosphorylation of mitogen activated protein (MAP) kinase. Melatonin is reported to inhibit MAP kinase activity (Hazerigg et al. 1999), which could be the another means by which melatonin suppresses TNF-α production. However, further studies in this direction are warranted to discover the role of MAP kinase inhibition by melatonin in suppressing lipopolysacharides-induced TNF-α production from macrophages. While the dose range of melatonin tested in in vitro experiments cannot be correlated with that of in vivo observations, it is useful for predicting other mecha-

nisms by which melatonin possibly suppresses the lipopolysacharides-induced TNF-α release by macrophages.

In the present study, we have not demonstrated a direct link between the inhibition of lipopolysacharides induced hyperalgesia by melatonin. However consequence of lipopolysacharides-induced sensitization of primary afferent or an established hyperalgesia is a result of activation of pro-inflammatory cytokines (Rietschell et al., 1994; Watkins et al., 1994). Any substances, which neutralize or inhibit the release of pro-inflammatory cytokines, were shown to reverse lipopolysacharides-induced hyperalgesia (Cunha et al., 1992; Ferreira et al., 1993; Lang et al., 1995; Watkins et al., 1995; Woolf et al., 1997). As in in vitro work, in vivo studies showed the ability of melatonin to reverse the rise in the lipopolysacharides-induced pro-inflammatory cytokine level, thereby preventing septic shock and reversing endotoxin-induced sickness behavior (Nava et al., 1997; Sacco et al., 1998; Raghavendra et al., 1999). Considering these findings, we hypothesize that melatonin attenuate the lipopolysacharides-induced central and peripheral hyperalgesia as a consequence of the inhibition of immune regulatory effect of lipopolysacharides.

Acknowledgements

The Senior Research Fellowship (V.R.) of the Council of Scientific and Industrial Research (CSIR), New Delhi, is gratefully acknowledged. The authors thank Morepen Laboratories, Parwano, India for generously donating melatonin used in this study.

References

Bauerle, P.A., Henkel, T., 1994. Functional and activation of NFkB in the immune system. Annu. Rev. Immunol. 12, 141–179.

Bulger, E.M., Garcia, I., Maier, R.V., 1998. Dithiocarbamates enhance tumor necrosis factor-α production by rabbit alveolar macrophages, despite inhibition of NF-kB. Shock 9, 397–405.

Chuang, J.I., Mohan, N., Meltz, M.L., Reiter, R.J., 1996. Effect of melatonin on the NFkB DNA binding activity in the rat spleen. Cell Biol. Int. 10, 687–692.

Costantino, G., Cuzzocrea, S., Marron, S., Caputi, A.P., 1998. Protective effects of melatonin in zymosan-activated plasma-induced paw inflammation. Eur. J. Pharmacol. 363, 57–63.

Cunha, F.Q., Poole, S., Lorenzetti, B.B., Ferreira, S.H., 1992. The pivotal role of tumor necrosis factor α in the development of inflammatory hyperalgesia. Br. J. Pharmacol. 107, 660–664.

Cuzzocrea, S., Zingarelli, B., Gilad, E., Hake, P., Salzman, A.L., Szabo, C., 1997. Protective effect of melatonin in carrageenan-induced models of local inflammations: relationship to its inhibitory effect on nitric oxide production and its peroxynitrite scavenging activity. J. Pineal Res. 23, 106–116.

Di Rosa, M., Radomski, M., Carnuccio, R., Moncada, S., 1990. Glucocorticoids inhibit the induction of nitric oxide synthase in macrophages. Biochem. Biophys. Res. Commun. 172, 1246–1252.

Dray, A., Bevan, S., 1993. Inflammation and hyperalgesia highlighting the team effort. Trends Pharmacol. Sci. 14, 287–290.

Ferreira, S.H., Lorenzetti, B.B., Poole, S., 1993. Bradykinin initiates cytokine-mediated inflammatory hyperalgesia. Br. J. Pharmacol. 110, 1227–1231.

Garcia-Perganeda, A., Guerrero, J.M., Rafii-El-Idrissi, M., Romero, P., Pozo, D., Calvo, J.R., 1999. Characterization of membrane melatonin receptor in mouse peritoneal macrophages: inhibition of adenylyl cyclase by a pertusis toxin-sensitive G protein. J. Neuroimmunol. 95, 85–94.

Gilad, E., Wong, H.R., Zingarelli, B., Virag, L., O'Connor, M., Salzman, A.L., Szabo, C., 1998. Melatonin inhibits expression of the inducible isoform of nitric oxide synthase in murine macrophages: role of inhibition of NFkappaB activation. FASEB J. 12, 685–693.

Golombek, P., Povet, P.D., Cardinali, D.P., 1995. Melatonin effects on behavior: possible mediation by the central GABAergic system. Neurosci. Biobehav. Rev. 20, 403–412.

Hazerigg, D.G., Thompson, M., Hastings, M.H., Morgan, P.J., 1999. Regulation of mitogen-activated protein kinase in the pars tuberalis of the ovine pituitary: interactions between melatonin, insulin growth factor-1, and forskolin. Endocrinology 137, 210–218.

Hylden, J.L.K., Thomas, D.A., Iadarola, M.J., Nahin, R.L., Dubner, R., 1991. Spinal opioid analgesic effects are enhanced in a model of unilateral inflammation/hyperalgesia: possible involvement of noradrenargic mechanisms. Eur. J. Pharmacol. 194, 135–143.

Kanaan, S.A., Garabedian, B.S., Haddad, J.J., Abdelnoor, A.M., Atweh, S.F., Jabbur, S.J., Saade, N.E., 1997. Effects of various analgesic and anti-inflammatory drugs on endotoxin-induced hyperalgesia in rats and mice. Pharmacology 54, 285–297.

Kanaan, S.A., Saade, N.E., Haddad, J.J., Abdelnoor, A.M., Atweh, S.F., Jabbur, S.J., Garabedian, B.S., 1996. Endotoxin-induced local inflammation and hyperalgesia in rats and mice: a new model for inflammatory pain. Pain 66, 373–379.

Lang, F., Robert, J.M., Boucrot, P., Welin, L., Petit, J.Y., 1995. New anti-inflammatory compounds that inhibit tumor necrosis factor production: probable interaction with protein kinase C activation. J. Pharmacol. Exp. Ther. 275, 171–176.

Liebmann, P.M., Wolfler, A., Felsner, P., Hofer, D., Schauenstein, K., 1997. Melatonin and the immune system. Int. Arch. Allergy Immunol. 112, 203–211.

Maestroni, G.J., Conti, A., 1991. Melatonin-immuno-opioid network: evidence for a physiological mechanism involving T cell-derived, immunoreactive β-endorphin and MET-enkaphalin binding to thymic opioid receptors. Int. J. Neurosci. 61, 289–298.

Missbach, M., Jagher, B., Sigg, I., Nayeri, S., Carlberg, C., Wiesenberg, I., 1996. Thiazolidine diones, specific ligands of the nuclear receptor retinoid Z receptor/retinoid acid receptor-related orphan receptor alpha with potent antiarthritic activity. J. Biol. Chem. 271, 3515–13522.

Mohan, N., Sadeghi, K., Reiter, R.J., Meltz, M.L., 1995. The neurohormone melatonin inhibits cytokine, mitogen and ionizing radiation induced NFkB. Biochem. Mol. Biol. Int. 37, 1063–1070.

Muller, J.M., Ziegler-Heitbrock, H.W., Baeucrle, P.A., 1993. Nuclear factor kappa B, a mediator of lipopolysaccharide effects. Immunobiology 187, 233–256.

Nava, F., Calpai, G., Facciola, G., Cuzzocrea, S., Giuliani, G., De Sarro, A., Caputi, A.P., 1997. Melatonin effects on inhibition of thirst and fever induced by lipopolysaccharide in rat. Eur. J. Pharmacol. 331, 267–274.

Novogrodsky, A., Vanichkin, A., Patya, M., Gazit, A., Osherov, N., Levitzki, A., 1994. Prevention of lipopolysaccharide-induced lethal toxicity by tyrosine kinase inhibitors. Science 264, 1319–1322.

Pozo, D., Delgado, M., Fernandez-Santos, J.M., Calvo, J.R., Gomariz, R.P., Martin-Lacave, I., Ortiz, G.G., Guerrero, J.M., 1997. Expression of mel_{1a}-melatonin receptor mRNA in T and B subsets of lymphocytes from rat thymus and spleen. FASEB J. 11, 466–473.

Quan, N., Stern, E.L., Whiteside, M.B., Herkenham, M., 1999. Induction of pro-inflammatory cytokine mRNAs in the brain after peripheral injection of subseptic doses of lipopolysaccharide in the rat. J. Neuroimmunol. 93, 72–80.

Raghavendra, V., Agrewala, J.N., Kulkarni, S.K., 1999. Role of centrally administered melatonin and inhibitors of COX and NOS in LPS-induced hyperthermia and adepsia. Prostagland. Leuk. Essent. Fatty Acids 60, 249–253.

Raghavendra, V., Kulkarni, S.K., 1999. Reversal of morphine tolerance and dependence by melatonin: possible role of central and peripheral benzodiazepine receptors. Brain Res. 834, 178–181.

Rietschell, E., Kirikae, T., Schade, U., Mamat, U., Schimidt, G., Loponow, H., Ulmer, A., Zahringer, U., Seydel, U., Padova, F., Schrier, M., Brade, H., 1994. Bacterial endotoxin: molecular relationships of structure to activity and function. FASEB J. 8, 217–225.

Rothwell, N.J., Hopkins, S.J., 1995. Cytokines and the nervous system: II. Actions and mechanisms of actions. Trends Neurosci. 18, 130–136.

Saade, N.E., Shihabuddin, L.S., Atweh, S.F., Jabbur, S.J., 1993. The role of previous nociceptive input in development of autotomy following cordotomy. Exp. Neurol. 119, 280–286.

Sacco, S., Aquilini, L., Ghezzi, P., Pinza, M., Guglielmotti, A., 1998. Mechanism of the inhibitory effect of melatonin on tumor necrosis factor production in vivo and vitro. Eur. J. Pharmacol. 343, 249–255.

Watkins, L.R., Wiertelak, E.P., Goehler, L.E., Smith, K.P., Martin, D., Maier, S.F., 1994. Characterization of cytokine-induced hyperalgesia. Brain Res. 654, 15–26.

Watkins, L.R., Goehler, L.E., Retton, J., Brewer, M.T., Maier, S.F., 1995. Mechanism of tumor necrosis factor-alpha (TNF-alpha) hyperalgesia. Brain Res. 692, 244–250.

Woolf, C.J., 1994. A new strategy for the treatment of inflammatory pain: prevention or elimination of central sensitization. Drugs 47, 1–9.

Woolf, C.J., Allehorne, A., Garabedian, S.B., Poole, S., 1997. Cytokine, nerve growth factor and inflammatory hyperalgesia; the contribution of tumor necrosis factor alpha. Br. J. Pharmacol. 121, 417–424.

Zimmerman, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.