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Pharmacology, Biochemistry and Behavior 74 (2003) 641–648
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Possible mechanism of action in melatonin attenuation of haloperidol-induced orofacial dyskinesia

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Received 10 July 2002; received in revised form 30 September 2002; accepted 6 November 2002

Abstract

Tardive dyskinesia (TD) is a late complication of prolonged neuroleptic treatment characterized by involuntary movements of the oral region. In spite of high incidence and much research, the pathophysiology of this devastating movement disorder remains elusive. Chronic treatment with neuroleptics leads to the development of abnormal oral movements in rats, referred to as vacuous chewing movements (VCMs). VCMs in rats are widely accepted as an animal model of TD. Rats chronically treated with haloperidol (1.5 mg/kg ip) significantly developed VCMs and tongue protrusions. Melatonin dose-dependently (1, 2, and 5 mg/kg) reversed the haloperidol-induced VCM and tongue protrusions frequencies. Biochemical analysis reveals that chronic haloperidol treatment significantly induced lipid peroxidation and decreased the forebrain glutathione (GSH) levels in the rats. Chronic haloperidol-treated rats also showed decreased levels of antioxidant defense enzymes, superoxide dismutase (SOD), and catalase. Coadministration of melatonin (1, 2, and 5 mg/kg) along with haloperidol significantly reduced the lipid peroxidation and restored the decreased GSH levels by chronic haloperidol treatment, and significantly reversed the haloperidol-induced decrease in forebrain SOD and catalase levels in rats. However, a lower dose of melatonin (1 mg/kg) failed to reverse chronic haloperidol-induced decreases in forebrain GSH, SOD, and catalase levels. In conclusion, melatonin could be screened as a potential drug candidate for the prevention or treatment of neuroleptic-induced orofacial dyskinesia.

Keywords: Haloperidol; Tardive dyskinesia; VCMs; Oxidative stress; Melatonin

1. Introduction

Haloperidol (HPD) is a widely used neuroleptic drug for the treatment of acute and chronic psychosis. The use of typical antipsychotics such as haloperidol is limited by their tendency to produce a range of extrapyramidal movement disorders such as parkinsonism, akathesia, dystonia, and tardive dyskinesia (TD). TD, which occurs in 20–40% of the patient population undergoing chronic neuroleptic therapy, is characterized by repetitive involuntary movements, usually involving the mouth, face, and tongue, and sometimes limb and trunk musculature (Egan et al., 1997; Casey, 2000; Kulkarni and Naidu, 2001). The most serious aspects of TD are that it may persist for months or years after drug withdrawal and, in some patients, it is irreversible. Due to its severity, high incidence, and potential irreversibility, TD is regarded as a major clinical and ethical issue in psychiatry. In spite of its high frequency of occurrence and known etiology, the pathophysiology of TD remains elusive.

Oxidative stress and products of lipid peroxidation are implicated in the etiopathology of TD (Cadet et al., 1986; Coyle and Puttfarcken, 1993; Andreassen and Jorgensen, 2000). Chronic treatment with neuroleptics is reported to increase free radical production and oxidative stress (Balijepalli et al., 2001). Elkashef and Wyatt (1999) have reported that rats with vacuous chewing movements (VCMs) had significantly higher thiobarbituric acid reactive substances (TBARs) in the striatum, suggesting increased lipid peroxidation and free radical production

Abbreviations: GSH, glutathione; SOD, superoxide dismutase; TD, tardive dyskinesia; VCMs, vacuous chewing movements.

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PII: S0091-3057(02)01051-1

in these animals. The chronic use of neuroleptics is also reported to cause decreases in the activity of antioxidant defense enzymes superoxide dismutase (SOD) and catalase (Cadet et al., 1987). Free radicals are thought to play a role in aging process, and age is one of the risk factors for the development of TD. Richardson et al. (1986) reported that there was a positive correlation between increasing age and the development of TD, further supporting the role of free radicals in the pathogenesis of TD. Vitamin E, an antioxidant and free radical scavenger, has been reported to be effective in the treatment of TD (Gupta et al., 1999; Soares and McGrath, 2000; Elkashef and Wyatt, 1999), suggesting the potential role of free radicals in the pathogenesis of TD.

Melatonin (*N*-acetyl-5-methoxytryptamine), the chief indolamine produced by the pineal gland, has been shown to be an effective antioxidant (Reiter et al., 2000, 2001) and free radical scavenger (Bandyopadhyay et al., 2000; Bromme et al., 2000). Melatonin is a broad-spectrum free radical scavenger and indirect antioxidant. Melatonin, apart from direct free radical scavenging activity, indirectly enhances antioxidative defense mechanisms by increasing the activities of several antioxidant enzymes (Reiter et al., 1997) and by stimulating the synthesis of another important intracellular antioxidant, glutathione (GSH) (Urata et al., 1999). Melatonin has been shown to be a more potent scavenger of hydroxyl radicals than GSH and mannitol (Tan et al., 1993) and a more potent scavenger of peroxyl radicals than GSH, vitamin E, and vitamin C (Pieri et al., 1994). In addition, it has been found to inhibit the production of TBARs in brain homogenates (Pierrefiche et al., 1993). Furthermore, melatonin reportedly suppressed lipid peroxidation induced by iron (Kabuto et al., 1998; Lin and Ho, 2000), lipopolysaccharides (Sewerynek et al., 1995a,b), nitric oxide (Escames et al., 1997), and kinate (Melchiorri et al., 1995). There is a substantial body of evidence for the protective effect of melatonin against DNA, lipids, and proteins (Gitto et al., 2001), which is the result of a number of endogenous and exogenous free radical-generating processes. Moreover, melatonin has been shown to inhibit cell death in those exposed to MPTP and β-amyloid peptides (Iacovitti et al., 1997; Ortiz et al., 2001; Pappolla et al., 2000). Melatonin, because of its small size and high lipophilicity, crosses biological membranes easily (Reiter, 1991), thus reaching all components of the cell. Melatonin has been shown to exert an antioxidant effect on dopaminergic neurons (Kim et al., 1998), as well as dopaminergic-modulating activities (Zispel et al., 1982), and thus may be efficacious in the treatment of TD. Melatonin is also reported to be effective in improving TD symptoms in humans (Shamir et al., 2000, 2001). However, the mechanism of this protective effect of melatonin against TD remains to be elucidated.

The aim of the present study is to evaluate the protective effect of melatonin on haloperidol-induced oral dyskinesia in rats—a potential animal model for TD.

2. Methods

2.1. Animals

Male Wistar rats, bred in the Central Animal House facility of the Panjab University and weighing between 180 and 220 g, were used. The animals were housed under standard laboratory conditions, maintained on a 12-h light/dark cycle, and had free access to food and water. Animals were acclimatized to laboratory conditions before the test. Each animal was used only once in the experiments. All experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by the Institutional Animal Ethics Committee and were conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

2.2. Induction of orofacial dyskinesia

Haloperidol (1.5 mg/kg ip) was given chronically to rats for a period of 21 days to induce oral dyskinesia (Sasaki et al., 1995; Naidu and Kulkarni, 2001a,b). All the behavioral assessments were carried out 24 h after the last dose of haloperidol.

2.3. Behavioral assessment of orofacial dyskinesia

On the test day, rats were placed individually in a small ($30 \times 20 \times 30$ cm) Plexiglas cage for the assessment of oral dyskinesia. Animals were allowed 10 min to get used to the observation cage before behavioral assessments. To quantify the occurrence of oral dyskinesia, hand-operated counters were employed to score tongue protrusion and vacuous chewing frequencies. In the present study, VCMs are referred to as single mouth openings in the vertical plane not directed toward a physical material. If tongue protrusion, and VCMs occurred during a period of grooming, they were not taken into account. Counting was stopped whenever the rat began grooming, and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. The behavioral parameters of oral dyskinesia were measured continuously for a period of 5 min. In all the experiments, the scorer was unaware of the treatment given to the animals.

2.4. Dissection and homogenization

On the 22nd day of haloperidol treatment, the animals were sacrificed by decapitation immediately after behavioural assessments. The brains were removed, and the forebrain was dissected out, rinsed with isotonic saline, and weighed. A 10% (wt/vol) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The post-nuclear fraction for catalase assay was obtained by centrifu-

gation of the homogenate at 1000 × *g* for 20 min at 4 °C, and for other enzyme assays by centrifugation of the homogenate at 12,000 × *g* for 60 min at 4 °C.

2.5. Lipid peroxidation assay

The quantitative measurement of lipid peroxidation in forebrain was performed according to the method of Wills (1966). The amount of malondialdehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm using the Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nanomoles of MDA per milligram of protein using the molar extinction coefficient of chromophore (1.56×10^5 M^{-1} cm^{-1}).

2.6. Estimation of reduced GSH

Reduced GSH in the forebrain was estimated according to the method of Ellman (1959). An amount of 0.75 ml of homogenate was precipitated with 0.75 ml of 4% sulphosalicylic acid. The samples were centrifuged at 1200 × *g* for 15 min at 4 °C. The assay mixture contained 0.5 ml of supernatant and 4.5 ml of 0.01 M (in 0.1 M phosphate buffer, pH 8.0) DTNB. The yellow color that developed was read immediately at 412 nm using the Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nanomoles of GSH per milligram of protein.

2.7. Enzyme assays

2.7.1. SOD activity

SOD activity was assayed according to the method of Kono (1978), wherein the inhibition of reduction of nitrozoblue tetrazolium (NBT) by the SOD is measured at 560 nm using the Perkin Elmer lambda 20 spectrophotometer. Briefly, the reaction was initiated by the addition of hydroxylamine hydrochloride to the reaction mixture containing the NBT and the postnuclear fraction of forebrain homogenate. The results were expressed as units per milligram of protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 50%.

2.7.2. Catalase activity

Catalase activity was assayed by the method of Luck (1971), wherein the breakdown of H_2O_2 was measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H_2O_2–phosphate buffer (1.25×10^{-2} M H_2O_2) and 0.05 ml of supernatant of the forebrain homogenate (10%), and the change in absorbance was recorded at 240 nm using the Perkin Elmer lambda 20 spectrophotometer. Enzyme activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.07). The results were expressed as micromoles of H_2O_2 decomposed per minute per milligram of protein.

2.8. Protein estimation

The protein content was measured according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.9. Drugs and treatment schedule

The following drugs were used in the present study. Haloperidol (Serenace inj.; Searle India, India) was diluted with distilled water. Melatonin (Morepen, India) was dissolved in 1% ethanol. All drugs were administered intraperitoneally in a constant volume of 0.5 ml/100 g body weight of rat. Animals were divided into four groups: saline/vehicle, vehicle/haloperidol, saline/melatonin, and melatonin/haloperidol. Haloperidol was administered once daily in the morning (0900 h) and this group also received vehicle for melatonin twice daily. Melatonin was given twice daily (0900 and 1700 h) for a period of 21 days, and behavioural assessments were carried out 24 h after the last dose of haloperidol. Drug doses were selected on the basis of previous studies conducted in our laboratory and those reported in the literature.

2.10. Statistical analysis

One specific group of rats was assigned to one specific drug treatment condition and each group comprised six rats ($n = 6$). All the values are expressed as means ± S.E.M. The data were analyzed using analysis of variance (ANOVA) followed by Dunnett's test. In all tests, the criterion for statistical significance was $P < .05$.

3. Results

3.1. Behavioral effects of chronic haloperidol or melatonin treatment in rats

Chronic haloperidol (1.5 mg/kg) treatment significantly increased the VCMs and tongue protrusions frequency in rats as compared to vehicle-treated controls. Melatonin (1, 2, and 5 mg/kg) alone did not induced any VCM or tongue protrusion (Table 1).

Table 1
Behavioral effects of chronic haloperidol or melatonin treatment in rats

Treatment [mg/kg]	VCMs/ 5 min	Tongue protrusions/5 min
Vehicle	4.2 ± 1.3	2.1 ± 0.3
Haloperidol (1.5)	58.3 ± 5.4 *	22.8 ± 3.0 *
Melatonin (1)	5.0 ± 1.6	2.1 ± 0.2
Melatonin (2)	6.1 ± 1.3	3.0 ± 0.7
Melatonin (5)	6.2 ± 1.3	2.3 ± 0.3

Values are expressed as mean ± S.E.M. of six animals.

* $P < .05$ as compared to vehicle-treated group (ANOVA followed by Dunnett's test).

Table 2
Biochemical effects of chronic haloperidol or melatonin treatment in rats

Treatment	Lipid peroxidation [nmol MDA/mg protein]	GSH [nmol/mg protein (% of control)]	SOD [U/mg protein (% of control)]	Catalase [μmol H_2O_2 decomposed/min/mg protein (% of control)]
Vehicle	1.3 ± 0.1	102.0 ± 3.4	100.0 ± 4.3	99.2 ± 7.2
Haloperidol (1.5)	3.6 ± 0.1 *	45.2 ± 6.2 *	37.4 ± 5.4 *	46.3 ± 6.2 *
Melatonin (1)	1.4 ± 0.1	98.3 ± 4.4	98.6 ± 5.2	99.7 ± 6.9
Melatonin (2)	1.3 ± 0.1	100.4 ± 4.1	97.3 ± 6.4	99.0 ± 4.6
Melatonin (5)	1.3 ± 0.1	101.3 ± 3.7	99.6 ± 4.1	101.7 ± 4.6

Values are expressed as mean ± S.E.M. of six animals in the case of lipid peroxidation and values are expressed as percent response of vehicle-treated control group in case of GSH, SOD, and catalase.
* $P < .05$ as compared to vehicle-treated group (ANOVA followed by Dunnett's test).

3.2. Biochemical effects of chronic haloperidol or melatonin treatment in rats

Chronic haloperidol-treated (1.5 mg/kg) animals showed decreased levels of GSH and increased levels of lipid peroxidation products as compared to vehicle-treated control animals. Chronic haloperidol-treated animals showed low levels of detoxifying enzymes such as SOD and catalase as compared to vehicle-treated control animals. Melatonin (1, 2, and 5 mg/kg) alone did not induce any biochemical alterations (Table 2).

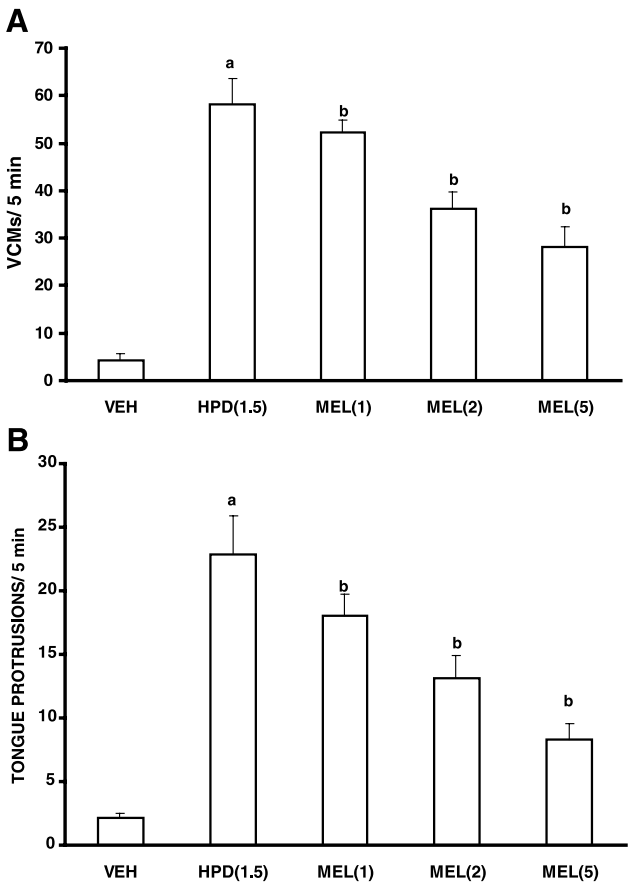

Fig. 1. Effect of coadministration of melatonin (MEL) on chronic haloperidol (HP)-induced VCMs (A) and tongue protrusions (B) in rats. Values expressed as mean ± S.E.M. [a]$P < .05$ as compared to vehicle-treated control group. [b]$P < .05$ as compared to haloperidol-treated group (ANOVA followed by Dunnett's test).

3.3. Effect of melatonin on haloperidol-induced VCMs and tongue protrusions

Chronic haloperidol treatment significantly increased the VCM and tongue protrusions frequency in rats as compared to vehicle-treated controls. Coadministration of melatonin dose-dependently (1, 2, and 5 mg/kg) suppressed the haloperidol-induced VCMs (Fig. 1A) and tongue protrusions (Fig. 1B).

3.4. Effect of melatonin on the forebrain MDA level in chronic haloperidol-treated rats

Chronic haloperidol treatment for 21 days induced lipid peroxidation as indicated by a significant raise in forebrain MDA levels as compared to vehicle-treated rats. Coadministration of melatonin (1, 2, and 5 mg/kg) along with haloperidol significantly reversed the extent of lipid peroxidation as compared to only haloperidol-treated rats (Table 3).

3.5. Effect of melatonin on the forebrain GSH levels in chronic haloperidol-treated rats

Statistical analysis of forebrain GSH levels showed a significant difference between vehicle-treated and haloper-

Table 3
Effect of coadministration of melatonin on chronic haloperidol-induced lipid peroxidation in the rat forebrain

Treatment [mg/kg]	Lipid peroxidation [nmol MDA/mg protein]
Vehicle	1.3 ± 0.1
Haloperidol (1.5)	3.6 ± 0.1*
Melatonin (1)	2.5 ± 0.1**
Melatonin (2)	2.6 ± 0.1**
Melatonin (5)	1.8 ± 0.1**

Values are expressed as mean ± S.E.M. of six animals.
* $P < .05$ as compared to vehicle-treated group (ANOVA followed by Dunnett's test).
** $P < .05$ as compared to haloperidol-treated group (ANOVA followed by Dunnett's test).

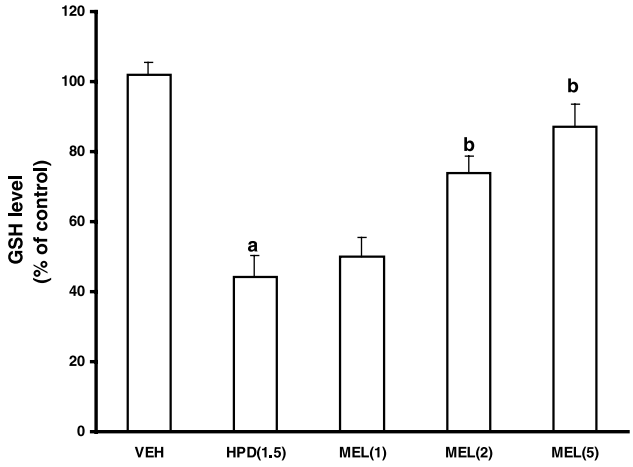

Fig. 2. Effect of coadministration of melatonin (MEL) on chronic haloperidol (HPD)-induced GSH depletion in rats. Values are expressed as percent response of vehicle-treated control group. Each value represents the mean value of six animals. [a]$P<.05$ as compared to vehicle-treated control group. [b]$P<.05$ as compared to haloperidol-treated group (ANOVA followed by Dunnett's test).

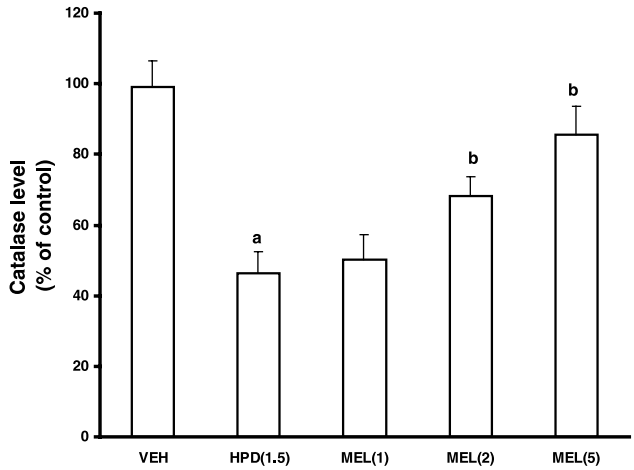

Fig. 4. Effect of coadministration of melatonin (MEL) on chronic haloperidol (HPD)-mediated depletion in the level of forebrain antioxidant enzyme catalase. Values are expressed as percent response of vehicle-treated control group. Each value represents the mean value of six animals. [a]$P<.05$ as compared to vehicle-treated control group. [b]$P<.05$ as compared to haloperidol-treated group (ANOVA followed by Dunnett's test).

idol-treated rats. Chronic administration of haloperidol significantly decreased the forebrain GSH levels. Coadministration of melatonin (2 and 5 mg/kg twice daily for 21 days) significantly reversed the haloperidol-induced (Fig. 2) decrease in the forebrain GSH levels. However, a lower dose of melatonin (1 mg/kg) failed to reverse the chronic haloperidol-induced decrease in forebrain GSH levels.

3.6. Effect of melatonin on the forebrain antioxidant enzyme levels in chronic haloperidol-treated rats

Chronic haloperidol-treated rats showed decreased levels of the antioxidant enzymes SOD and catalase in their forebrain homogenates. Coadministration of melatonin (2 and 5 mg/kg) significantly reversed the haloperidol-induced decrease in forebrain SOD (Fig. 3) and catalase (Fig. 4) levels as compared to only haloperidol-treated rats. However, a lower dose of melatonin (1 mg/kg) failed to reverse chronic haloperidol-induced decreases in forebrain SOD and catalase levels.

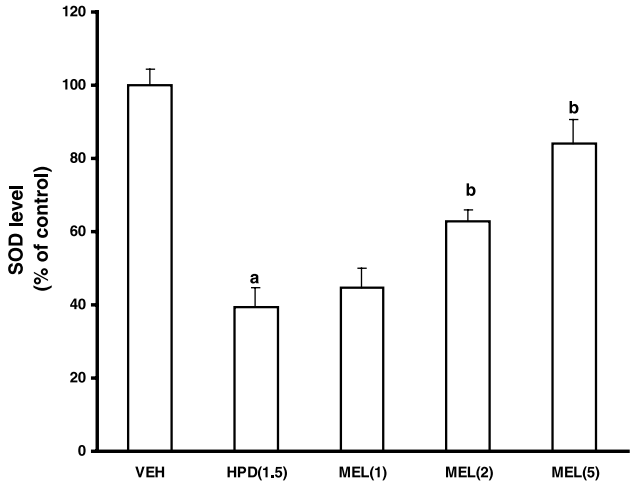

Fig. 3. Effect of coadministration of melatonin (MEL) on chronic haloperidol (HPD)-mediated depletion in the level of forebrain antioxidant enzyme SOD. Values are expressed as percent response of vehicle-treated control group. Each value represents the mean value of six animals. [a]$P<.05$ as compared to vehicle-treated control group. [b]$P<.05$ as compared to haloperidol-treated group (ANOVA followed by Dunnett's test).

4. Discussion

In the present study, chronic haloperidol-treated animals showed increased frequencies of VCMs and tongue protrusions as compared to vehicle-treated control animals. Chronic treatment with melatonin significantly reversed the-induced VCMs and tongue protrusions in a dose-dependent fashion.

Numerous reports indicate that an excessive production of free radicals is associated with chronic neuroleptic use and might contribute to the onset of TD and other movement disorders, such as dystonia and parkinsonism (Cadet et al., 1986). This effect can be related, at least in part, to a reduction in specific endogenous antioxidant mechanisms, such as a decrease in GSH levels (Shivakumar and Ravindranath, 1993) and low levels of antioxidant defense enzymes such as SOD and catalase (Elkashef and Wyatt, 1999).

The molecular mechanisms by which neuroleptics increase oxygen free radical production are unknown. Neuroleptics act by blocking dopamine receptors (Creese et al., 1976). Such blockade results in increased dopamine turnover, which in turn could conceivably lead to an increased production of hydrogen peroxide, resulting in oxidative stress (Cohen and Spina, 1988; Elkashef and Wyatt, 1999). Oxygen free radicals are also reported to diminish the dopamine

transporter function, further increasing the extracellular dopamine levels (Fleckenstein et al., 1997).

However, this does not seem to be the only mechanism responsible for the GSH/ATP depletion observed during haloperidol treatment (Vairetti et al., 1999). Very recently, using rat primary cortical neurones and the mouse hippocampal cell line HT-22, Sagara (1998) showed that haloperidol causes a sequence of cellular alterations that leads to cell death, and that the production of reactive oxygen species (from the mitochondria but not from the metabolism of catecholamines) is an integral part of this cascade. Thus, the possibility of a direct interaction with specific membrane components exists. Cohen and Zubenko (1985) have in fact demonstrated that striatal cell membranes of rats chronically treated with neuroleptics exhibit abnormal physicochemical properties. It is conceivable that the changes in membrane properties may be related to free radical production. Another possibility is that neuroleptics suppress the activity of certain detoxifying enzymes, leaving cells unprotected especially if basal enzyme activity is low or the free radical scavenging mechanisms are less effective. Free radicals are highly reactive with specific cellular components and have cytotoxic properties (Raveindranath and Reed, 1990), and neuronal loss in the striatum has been reported in animals treated chronically with neuroleptics (Nielsen and Lyon, 1978).

Neuroleptics may also have a direct cytotoxic effect via the production of toxic metabolites (Gorrod and Fang, 1993; Wright et al., 1998). Galili et al. (2000) reported that the direct neurotoxic effects of haloperidol and its metabolites on mouse neuronal cultures and PC-12 cells were reversed by antioxidants. Burkhard et al. (1993) reported that, like MPP^+, the metabolites of haloperidol, chlorpromazine, and thioxanthine inhibited Complex I of the electron transport chain; clozapine was also found to inhibit Complex I, but at a much higher concentrations—this might be one of the possible mechanisms for the development of TD.

In the present study, chronic haloperidol-treated animals showed decreased levels of GSH and increased levels of lipid peroxidation products as compared to vehicle-treated control animals. Chronic haloperidol-treated animals showed low levels of detoxifying enzymes such as SOD and catalase as compared to vehicle-treated control animals, suggesting a possible induction of free radical generation by chronic haloperidol treatment. Melatonin dose-dependently decreased the elevated level of lipid peroxidation products in haloperidol-treated animals, also elevated the cellular defense mechanisms such as GSH, and also induced the production of SOD and catalase, further suggesting the role of free radical in the pathophysiology of haloperidol-induced orofacial dyskinesia and possible antioxidant action of melatonin.

The mechanism by which melatonin prevents haloperidol-induced oxidative damage and orofacial dyskinesia is unclear. Melatonin attenuated both reserpine-induced and age-induced orofacial movements and decreased the reserpine-induced striatal lipid peroxidation. Continuous exposure to light increased spontaneous as well as reserpine-induced orofacial movements, indicating that endogenous melatonin may be involved in this movement disorder (Abilio et al., 2002). Melatonin was reported to prevent the oxidative stress caused by transition metal-accelerated catecholamine auto-oxidation (Miller et al., 1996). Melatonin has been reported to activate antioxidant defensive enzymes, including increasing mRNA levels of SOD (Kolter et al., 1998) and GSH peroxidase (Barlow-Walden et al., 1995), GSH reductase, and catalase (Reiter, 2000; Gepdiremen et al., 2000). Another reported mechanism of melatonin action involves free radical scavenging (Tan et al., 1993). Moreover, a recent study has shown that melatonin binds several metals, including iron (III) (Limson et al., 1998), and this effect may attenuate a biological Fenton's reaction that results in oxidative damages. It also prevents singlet oxygen toxicity (Cagnoli et al., 1995) and scavenges peroxynitrite anions (Cuzzocrea et al., 1997), both of which damage neuronal tissue. Melatonin also inhibits nitric oxide synthase (NOS), a pro-oxidative enzyme (Pozo et al., 1997).

Several classical antioxidants are known pro-oxidants (Aruoma, 1997). Melatonin has been shown to be devoid of pro-oxidative activity in a variety of experimental models (Reiter, 1998; Tan et al., 2000). Melatonin is a highly effective $OH^{\cdot}$ scavenger (Tan et al., 1993; Bandyopadhyay et al., 2000; Bromme et al., 2000). Indeed, each molecule of melatonin scavenges two $OH^{\cdot}$ with the formation of a stable end product, cyclic-3-hydroxy-melatonin, which is excreted in the urine (Tan et al., 1998). Melatonin also scavenges H_2O_2, during which it is metabolized to *N*-acetyl-N^2-formyl-5-methoxy-kynuramine (AFMK) (Tan et al., 2000). Since melatonin is sacrificed and does not participate in redox cycling after scavenging free radicals, not only does melatonin not consume the basic cellular reductive force GSH, but it also preserves or even increases the content of GSH in tissues (Sewerynek et al., 1995a,b,c; Urata et al., 1999). Several classic antioxidants are known pro-oxidants. Thus, melatonin is a suicidal or terminal antioxidant offering a greater advantage over conventional antioxidants.

The activation of excitatory amino acid receptors leads to increased intracellular calcium followed by activation of protein kinases, phospholipases, proteases, nitric oxide synthesis, impaired mitochondrial function, and generation of free radicals (Schulz et al., 1995). Melatonin counteracts the in vitro destructive effects of NMDA by preventing an excessive accumulation of free radicals (Cazevieille et al., 1997). As glutamate, calcium, and NO are implicated in the pathophysiology of TD (Naidu and Kulkarni, 2001c), the beneficial effect of melatonin might be due to its antagonistic effect on NMDA-mediated excitotoxicity.

Accordingly, one or several of these actions of melatonin may contribute to the suppression of haloperidol-induced oxidative injury and orofacial dyskinesia.

In conclusion, the findings of the present study strongly suggest that free radical toxicity and oxidative stress play an important role in the pathogenesis of orofacial dyskinesia,

and that melatonin could be used for the prevention or treatment of neuroleptic-induced orofacial dyskinesia.

Acknowledgements

The Senior Research Fellowship (P.S. Naidu) of the Council of Scientific and Industrial Research (CSIR), New Delhi, and the technical assistance provided by Ms. Saraswati Gupta are gratefully acknowledged.

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