Psychopharmacology (2002) 161:340–347
DOI 10.1007/s00213-002-1081-7

ORIGINAL INVESTIGATION

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Effects of melatonin on orofacial movements in rats

Received: 16 July 2001 / Accepted: 20 February 2002 / Published online: 26 April 2002

Abstract *Rationale:* While reserpine-induced oral movements (OM), an animal model of tardive dyskinesia, are more persistent in old than in adult rats, old animals present spontaneous OM, which are phenomenologically similar to those presented by reserpine-treated adult rats. We postulate that these OM may be the result of oxidative stress induced by both age and reserpine treatment.*Objectives:* We intended to determine the preventative effects of exogenous melatonin (one of the most important endogenous antioxidants) as well as suppression of endogenous melatonin via continuous exposure to light on reserpine- or age-induced OM in rats. *Methods:* Adult (4 months of age) male Wistar rats were repeatedly treated with saline or melatonin (5 mg/kg, IP) and saline or reserpine and kept under a 12-h light/dark cycle for quantification of reserpine-induced OM as well as oxidative stress (via quantification of lipid peroxidation). To verify the effects of endogenous melatonin suppression on reserpine-induced OM, adult rats were repeatedly treated with saline or reserpine and continuously exposed to light. To verify the effects of exogenous melatonin on age-induced OM older (20 months of age) rats were long-term treated with saline or melatonin and kept under a 12-h light/dark cycle. *Results:* Melatonin attenuated both reserpine- and age-induced OM. Reserpine enhanced striatal lipid peroxidation, that was prevented by melatonin co-administration. Continuous exposure to light increased spontaneous as well as reserpine-induced OM, indicating that endogenous melatonin may be involved in this movement disorder. *Conclusions:* We suggested that melatonin attenuates both reserpine- and age-induced OM in rats.

Keywords Melatonin · Dopamine · Orofacial movement · Tardive dyskinesia · Oxidative stress · Aging

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Introduction

Tardive dyskinesia (TD) is a syndrome characterized by repetitive involuntary movements, usually involving mouth, face and tongue and sometimes limb and trunk musculature. This syndrome is a late-onset adverse effect of prolonged administration of classical neuroleptic drugs. A major advantage of the new, so called atypical antipsychotic drugs is their lower liability for acute extrapyramidal side-effects as well as indications of a reduced risk of developing TD. Patients should find these new drugs more tolerable and acceptable, although whether or not this will translate into better compliance in the longer term has yet to be established (Barnes and Spence 2000). In all probability, TD will be with us for at least the immediate future. Indeed, about 20–30% of conventional neuroleptic-treated patients present this syndrome that can last for years, being even irreversible in some cases (Yassa and Nair 1988).

TD has been suggested to be a consequence of dopamine receptor supersensitivity in the nigrostriatal system (Klawans 1973). In rats, abrupt withdrawal from long-term treatment with several dopamine blockers (Frussa-Filho and Palermo-Neto 1990, 1991; Queiroz and Frussa-Filho 1997; Abílio et al. 1999) enhanced not only general activity in an open-field but also the stereotypy response to apomorphine. This behavioral supersensitivity is thought to result from receptor site proliferation in response to chronic dopamine receptor blockade (Burt et al. 1977), and has been proposed as a potential model for neuroleptic-induced TD in humans (see Palermo-Neto and Frussa-Filho 2001 for review).

Although the hypothesis of dopamine supersensitivity has dominated conceptual approaches to the study of

TD, some fundamental observations do not seem to support this hypothesis (Gerlach 1985; Wolfarth and Ossowska 1989; Casey 1995). One of the most important flaws is related to the effects of age on TD/dopaminergic supersensitivity. In this regard, age is the single most frequently implicated risk factor for TD, increasing both the risk of developing TD and the severity and persistence of the condition (Wolfarth and Ossowska 1989). Conversely, old animals have a diminished capacity to develop both behavioral supersensitivity to the apomorphine-induced stereotypy and dopamine receptor upregulation after chronic treatment with neuroleptics (Waddington and Gamble 1980; Randall et al. 1981).

Recently, Neisewander et al. (1994) have suggested that reserpine-induced oral movements may provide an animal model of TD. Indeed, rats withdrawn from treatment with this monoamine-depleting agent for at least 24 h develop orofacial movements (tongue protrusion, twitching of the facial musculature and vacuous chewing movements) (Neisewander et al. 1991a, 1991b, 1994, 1996; Vital et al. 1997; Bergamo et al. 1997; Sussman et al. 1997; Queiroz et al. 1998; Queiroz and Frussa-Filho 1999). In this regard, vacuous chewing movements induced by long-term neuroleptic treatment have also been extensively studied (see Waddington 1990). However, it is still debatable whether neuroleptic-induced purposeless chewing movements are a model of TD or of acute dystonia. In fact, the latter possibility is supported by the observation that the number of vacuous chewing movements increases just after the first dose of neuroleptic, whereas withdrawal of neurolpetic treatment is followed by a quick decrease to control levels (see Wolfarth and Ossowska 1989). Concerning these critical issues for evaluating animal models of TD, reserpine-induced orofacial movements (or at least reserpine-induced tongue protrusion) seem to be a better model of TD. Indeed, acute dystonia frequently develops after the first dose of neuroleptic, whereas reserpine produces a decrease in tongue protrusions in animals observed 6 h after the first injection (Neisewander et al. 1991a, 1994). In addition, reserpine-induced orofacial movements appear late during the course of administration at low doses and persists for a long time following termination of administration (Neisewander et al. 1994). Although reserpine is not classified as a neuroleptic, it has been used as an antipsychotic agent and has been associated with the development of tardive dyskinesia (Uhrbrand and Faurbye 1960). These reserpine-induced orofacial movements (especially tongue protrusion frequency) in rats also have other features that are consistent with TD. As with TD (Wolfarth and Ossowska 1989), reserpine-induced oral movements are attenuated by a D_2 dopamine receptor antagonist (Neisewander et al. 1991b), exacerbated by dopamine agonists such as amphetamine, and seem to be mediated, at least in part, by the nigrostriatal dopaminergic system, since they are attenuated by nigrostriatal 6-hydroxydopamine lesions (Neisewander et al. 1996). Finally, consistent with the clinical situation, we have recently verified that in old rats, the persistence of reserpine-induced orofacial movements was increased when compared to adult animals (Bergamo et al. 1997).

Whereas increased oxidative stress with cumulative free-radical damage is a well known feature of the aging brain (Benzi and Moretti 1995), the proposal that TD is due to a neurotoxic effect of the free-radical byproducts from catecholamine metabolism in the basal ganglia has been receiving considerable interest (Lohr 1991; Casey 1995; Andreassen and Jørgensen 2000). Specifically, neuroleptic drugs, by blocking dopamine receptors, cause a secondary increase in turnover and metabolism of dopamine, which may lead to increased formation of dopamine quinones as well as of hydrogen peroxide through the activity of MAO (Lohr 1991). In support of this "free-radical hypothesis" of TD, in vitro and in vivo studies have shown that neuroleptic drugs induce oxidative stress and cell death (Behl et al. 1996; Kleeb et al. 1999). Furthermore, clinical studies have found increased levels of lipid peroxidation byproducts in blood or cerebrospinal fluid of tardive dyskinesia compared to non-tardive dyskinesia patients (Lohr et al. 1990).

Melatonin, the main hormone produced by the pineal gland, seems to be related not only to a wide variety of bodily functions (Reiter 1996) but also to the aging process (Pierpaoli and Regelson 1994) and is considered to be an important free radical scavenger (for review, see Reiter 1998). Its production occurs during the dark phase of the circadian cycle (Arendt 1988) and is inhibited by exposure to light (Roberts 1995). In addition, in a very recent study, Raghavendra et al. (2001) verified that acute administration of melatonin attenuates reserpine-induced vacuous chewing movements in adult rats.

The main purpose of the present study was to investigate the preventative effects of melatonin on reserpine- as well as on age-induced orofacial movements. To address this question, we have examined: 1) the effects of long-term melatonin administration and of endogenous melatonin suppression on the development of reserpine-induced orofacial movements in 4-month-old rats; 2) the effects of long-term reserpine administration concomitant or not with melatonin administration on striatal oxidative stress through the quantification of lipid peroxidation; and 3) the effects of exogenous melatonin on orofacial movements presented by older (20-month-old) animals.

Materials and methods

Healthy 4-month-old (weighing approximately 320 g) and 20-month-old (weighing approximately 400 g) male Wistar EPM-1 rats, born and raised under our laboratory conditions, were used. The animals were housed under conditions of controlled temperature (22–23°C) and under a 12-h light/dark cycle (LD) with lights on at 7:00 a.m. or continuously exposed to light (LL). Food and water were available ad libitum throughout the experiment. Animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

Drugs

Reserpine (Sigma-Aldrich, St Louis, Mo., USA) and melatonin (Sigma-Aldrich) were freshly diluted in distilled water. Reserpine was diluted in glacial acetic acid and melatonin was suspended in Tween 80 (3%). Saline+glacial acetic acid and saline+Tween 80 were used as reserpine and melatonin control solutions, respectively. Reserpine was administered subcutaneously whereas melatonin was administered IP in volumes not exceeding 1 ml/kg body weight. Melatonin dose (5 mg/kg) was selected on the basis of pilot experiments and reserpine dose (0.1 mg/kg) on the basis of literature reports (Neisewander et al. 1994).

Measurements of orofacial movements

The animals were observed for tongue protrusion frequency and duration of twitching of the facial musculature in wire mesh cages (16×30×19 cm) without food and water. Hand-operated counters and stopwatches were used to quantify these parameters, respectively. Tongue protrusion was defined as a visible extension of the tongue outside of the mouth and not directed at anything. Individual tongue protrusions during a bout of oral movements were each preceded by visible retraction of the tongue. Twitching of the facial musculature was defined as the sum of the duration of all continuous jaw tremors that lasted 3 s or more. If tongue protrusion or twitching of the facial musculature occurred during a period of grooming, they were not taken into account. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral movements when the animal faced away from the observer. To avoid differences in the behavior of experimental and control groups of rats due to circadian changes, experimental and control observations were alternated. The observations were made by three observers, who were blind to the animals' group assignment. The observation criteria were not subjective, since an excellent inter-observer agreement was found in previous pilot experiments (Pearson's correlation=0.98 for tongue protrusion and for twitching of the facial musculature).

Measurement of striatal lipid peroxidation

Lipid peroxidation measurement was performed by quantification of malondialdehyde (MDA), the most abundant product arising from this oxidative process (Kagan 1988). Striata were homogenized in 0.1 M phosphate buffer. Duplicates of each sample were used to determine malondialdehyde by measurement of fluorescent product formed from the reaction of this aldehyde with thiobarbituric acid, as described by Tanizawa et al. (1981). The results are expressed as nmol MDA/g tissue calculated by plotting the obtained fluorescence (excitation at 315 nm, emission at 553 nm) against a MDA concentration standard curve.

Measurement of serum melatonin level

Melatonin assay levels were performed in duplicate (500 µl aliquots of samples collected at approximately 0000 hours) by radioimunoassay (Bühlmann Laboratories AG, Allschwil, Switzerland). The limit of detection was 0.5 pg/ml. The intra-assay coefficient of variation was 9.8% (1.9 pg/ml) and 7.1% (21.8 pg/ml). The inter-assay coefficient of variation was 16.3% (2.3 pg/ml) and 7.2% (18.8 pg/ml). The results are expressed as pg melatonin/ml serum.

Procedure

Experiments 1 and 2

For experiment 1, 4-month-old rats were divided at random into five groups kept under a 12-h light/dark cycle: S+S-S, S+R-S, M+S-S, M+R-S and M+R-M. For experiment 2, 4-month-old rats were divided at random into two groups kept under a 12-h light/dark cycle: S+S-S, M+S-M. The animals of both experiments were injected once daily with saline (S) or 5.0 mg/kg melatonin (M) for 19 days and, every other day with saline or 0.1 mg/kg reserpine (R) 30 min after these injections. On day 20 after the beginning of the pharmacological treatment (24 h after reserpine or saline injection on day 19), the animals were injected with saline (-S) or 5.0 mg/kg melatonin (-M) and 1 h later the frequency of tongue protrusion and duration of twitching of the facial musculature were measured continuously for 15 min.

Experiment 3

Four-month-old rats were divided at random into four groups kept under a 12-h light/dark cycle: S+S-S, S+R-S, M+R-S and M+R-M. The animals were injected once daily with saline (S) or 5.0 mg/kg melatonin (M) for 19 days and, every other day with saline or 0.1 mg/kg reserpine (R) 30 min after these injections. On day 20 after the beginning of the pharmacological treatment (24 h after reserpine or saline injection on day 19), the animals were injected with saline (-S) or 5.0 mg/kg melatonin (-M) and 1 h later they were killed for quantification of striatal lipid peroxidation.

Experiment 4

In order to demonstrate that under our experimental conditions the light/light cycle would be effective in reducing melatonin levels, 4-month-old rats were divided at random into two groups that were kept for 24 h under a 12-h light/dark cycle (LD) or under a 24-h light/light cycle (LL). After this period of time, animals were killed by decapitation and blood samples were collected for quantification of serum melatonin levels.

Experiment 5

Four-month-old rats were divided at random into four groups: two control and two experimental groups. The control groups were injected every other day for 19 days with saline and were kept under a 12-h light/dark cycle (SAL LD) or under a 24-h light/light cycle (SAL LL: this schedule was started 6 days before the beginning of the pharmacological treatment), respectively. The two experimental groups were injected every other day for 19 days with 0.1 mg/kg reserpine and were kept under a 12-h light/dark cycle (RES LD) or under a 24 h light/light cycle (RES LL: starting 6 days before the beginning of pharmacological treatment), respectively. On day 20 after the beginning of the pharmacological treatment (24 h after reserpine or saline injection on day 19) the frequency of tongue protrusion and the duration of twitching of the facial musculature were measured continuously for 15 min.

Experiment 6

Four-month-old and 20-month-old rats were kept under a 12-h light/dark cycle. Four-month-old rats were injected with saline (SAL) and older rats (20-month-old) were divided at random into two groups injected with saline or 5.0 mg/kg melatonin (MEL). All animals were injected once daily for 5 months. One hour after the last injection, the frequency of tongue protrusion and the duration of twitching of the facial musculature were measured continuously for 15 min.

Statistical analysis

Data were treated by two-way or by one-way analysis of variance (ANOVA) followed by Duncan's test. For comparisons between only two groups the Student *t*-test was performed. A probability of $P<0.05$ was considered to show significant differences for all comparisons made.

Results

Experiments 1 and 2

Figure 1 shows the frequency of tongue protrusion (Fig. 1A) and duration of twitching of the facial musculature (Fig. 1B) of 4-month-old rats kept under the LD cycle treated with saline or reserpine and saline or melatonin and challenged with saline or melatonin. Analysis of variance revealed statistical differences for both behavioral measures [$F(4,42)=6.31$, $P<0.001$, $F(4,42)=3.48$, $P<0.05$, respectively].

When compared to the S+S-S group, the S+R-S group presented a significant increase in the frequency of tongue protrusion (Fig. 1A) and in duration of twitching of the facial musculature (Fig. 1B). The M+R-S group, when compared to the S+S-S group, presented a significant increase in the frequency of tongue protrusion (Fig. 1A), but did not show any differences in the duration of twitching of the facial musculature (Fig. 1B). When compared to the S+R-S group, the M+R-S group presented a significantly lower duration of twitching of the facial musculature (Fig. 1B). The M+R-M group, when compared to the S+S-S group, did not show any statistical difference in the frequency of tongue protrusion (Fig. 1A) or duration of twitching of the facial musculature (Fig. 1B), but when compared to the S+R-S group presented a significantly lower frequency of tongue protrusion (Fig. 1A) and duration of twitching of the facial musculature (Fig. 1B). Finally, there were no significant differences in the frequency of tongue protrusion or in the duration of facial twitching between the M+S-S and the S+S-S groups. In this respect, experiment 2 demonstrated that the frequency of tongue protrusion as well as the duration of facial twitching of the M+S-M group (0.91±0.39 and 4.00±2.80, respectively) did not differ from those presented by the S+S-S group (2.18±0.76 and 8.45±4.59, respectively) [$t(20)=1.49$ and $t=0.83$ for tongue protrusion and facial twitching, respectively]. On the basis of these results considered as a whole, although melatonin was ineffective per se in modifying spontaneous orofacial movements, melatonin co-treatment was able to attenuate the reserpine-induced increase in orofacial movements.

Experiment 3

Figure 2 shows the level of striatal lipid peroxidation of 4-month-old rats kept under the LD cycle treated with saline or reserpine and saline or melatonin and challenged with saline or melatonin. Analysis of variance revealed statistical differences [$F(3,20)=3.67$, $P<0.05$]. The S+R-S group presented an increase in lipid peroxidation when compared to the S+S-S group. Melatonin treatment was able to prevent this reserpine-induced increase in lipid peroxidation. Indeed, the M+R-S and the M+R-M groups presented a decrease in lipid peroxidation when compared to the S+R-S group without showing any difference when compared to the S+S-S group.

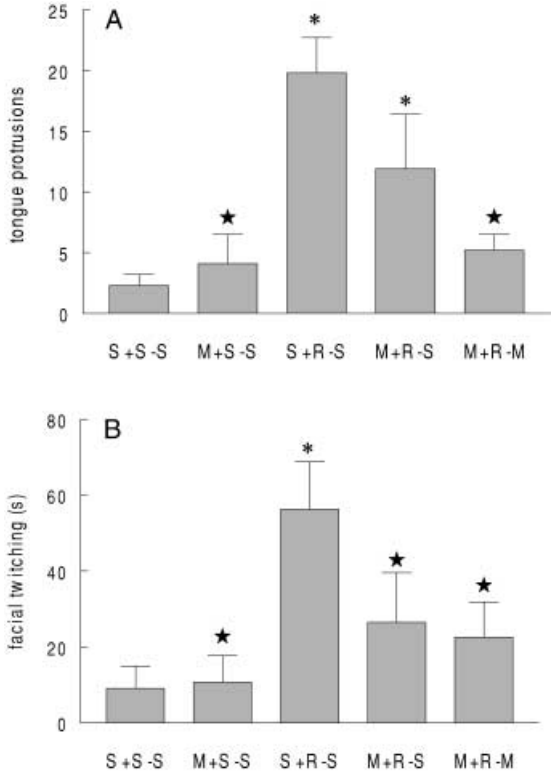

Fig. 1 Effects of daily injections of 5.0 mg/kg melatonin (*M*) or saline (*S*) concomitant with alternate day injections of 0.1 mg/kg reserpine (*R*) or saline (*S*), for 19 days, on the frequency of tongue protrusion (**A**) and duration of twitching of the facial musculature (**B**) of 4-month-old rats kept under a 12-h light/dark cycle. One hour before observation, the animals were injected with saline (-*S*) or 5.0 mg/kg of melatonin (-*M*). Data are reported as the mean±SEM. *Asterisks*, $P<0.05$ compared to S+S-S group; *stars*, $P<0.05$ compared to S+R-S group. Analysis of variance followed by Duncan's test

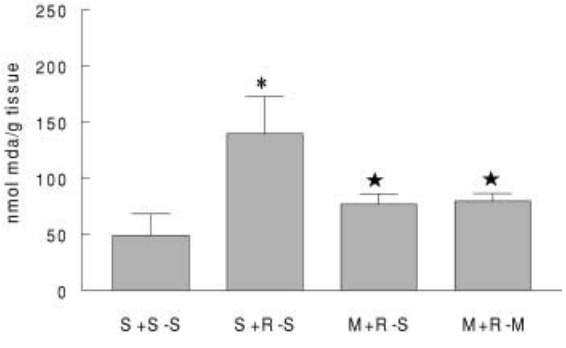

Fig. 2 Effects of daily injections of 5.0 mg/kg melatonin (*M*) or saline (*S*) concomitant with alternate day injections of 0.1 mg/kg reserpine (*R*) or saline (*S*), for 19 days, on striatal level of lipid peroxidation of 4-month-old rats kept under a 12-h light/dark cycle. One hour before death, the animals were injected with saline (-*S*) or 5.0 mg/kg melatonin (-*M*). Data are reported as the mean±SEM. *Asterisks*, $P<0.05$ compared to S+S-S group; *stars*, $P<0.05$ compared to S+R-S group. Analysis of variance followed by Duncan's test

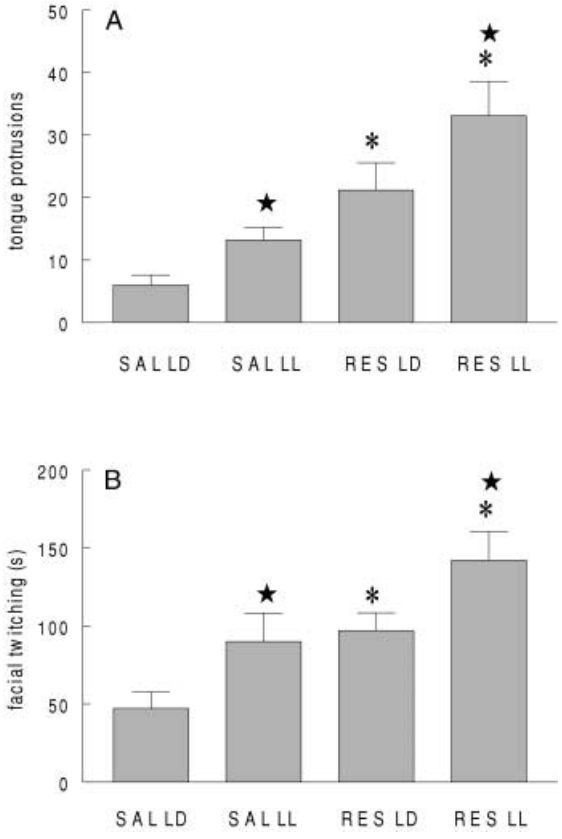

Fig. 3 Effects of alternate day administration of 0.1 mg/kg of reserpine (*RES*) or saline (*SAL*), for 19 days, on the frequency of tongue protrusion (**A**) and duration of twitching of the facial musculature (**B**) in 4-month-old rats kept under a 12-h light/dark cycle (*LD*) or a 24-h light/light cycle (*LL*). Data are reported as the mean±SEM. Two-way analysis of variance revealed the following effects: *asterisks*, $P<0.05$ for treatment effect (comparison between SAL and RES groups kept under the same cycle); *stars* $P<0.05$ for light-cycle effect (comparison between LD and LL groups submitted to the same treatment)

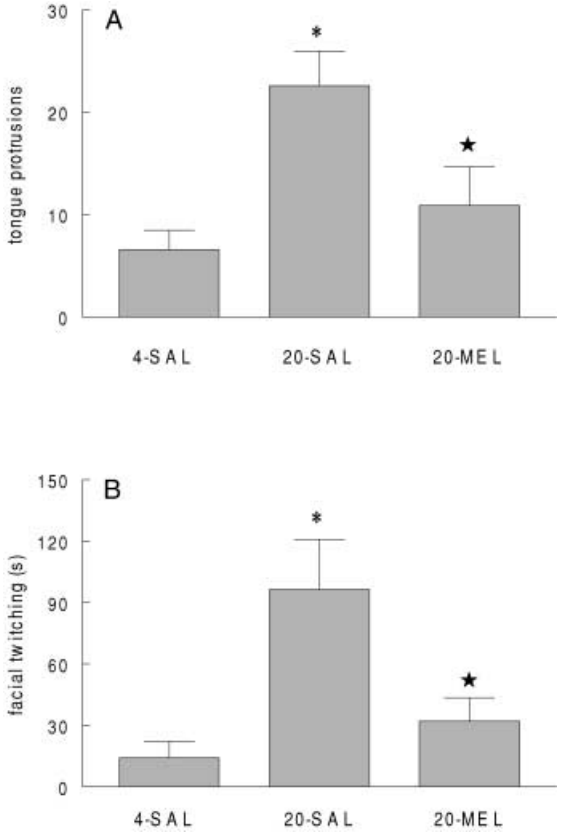

Fig. 4 Effects of daily injection of 5.0 mg/kg melatonin (-*MEL*) or saline (-*SAL*) for 5 months on the frequency of tongue protrusion (**A**) and duration of twitching of the facial musculature (**B**) presented by 4-month-old (4) or 20-month-old (20) rats kept under a 12-h light/dark cycle. Data are reported as the mean±SEM. *Asterisks* $P<0.05$ compared to the 4-SAL group. *Stars*, $P<0.05$ compared to the 20-SAL group. Analysis of variance followed by Duncan's test

Experiment 4

This experiment shows the level of serum melatonin of 4-month-old rats kept for 24 h under the LD or LL cycle. Continuous exposure to light was able to reduce serum melatonin level [$t(17)=2.53$, $P<0.05$]. Indeed, serum melatonin level of the LL group (10.00±0.93 pg/ml serum) was significantly lower than that of the LD group (15.24±0.93 pg/ml serum).

Experiment 5

Figure 3 shows the frequency of tongue protrusion (Fig. 3A) and duration of twitching of the facial musculature (Fig. 3B) of reserpine- or saline-treated 4-month-old animals kept under the LD or the LL cycle. Two-way analysis of variance with treatment and light-cycle as factors revealed significant treatment and light-cycle effects for tongue protrusion [$F(1,34)=22.49$, $P<0.001$, $F(1,34)=6.69$, $P<0.05$, respectively] and for twitching of the facial musculature [$F(1,34)=12.01$, $P<0.001$, $F(1,34)=8.87$, $P<0.01$, respectively]. No significant treatment×light-cycle interactions were found. Thus, concerning the treatment effect, reserpine increased orofacial movements in rats kept under both cycles, and concerning the light-cycle effect, exposure to the LL cycle increased the orofacial movements in saline- as well as in reserpine-treated animals.

Experiment 6

Figure 4 shows the frequency of tongue protrusion (Fig. 4A) and duration of twitching of the facial musculature (Fig. 4B) of saline-treated 4-month-old animals and of saline- or melatonin-treated 20-month-old animals kept under the LD cycle. Analysis of variance revealed statistical differences for both parameters [$F(2,19)=7.35$, $P<0.005$, $F(2,19)=7.51$, $P<0.005$, respectively].

Older rats presented spontaneous orofacial movements. Thus, saline-treated old rats presented an increase

in the frequency of tongue protrusion (Fig. 4A) and duration of twitching of the facial musculature (Fig. 4B) when compared to saline-treated 4-month-old animals. Melatonin treatment attenuated the spontaneous orofacial movements presented by older animals. Thus, melatonin-treated older animals, when compared to saline-treated older animals, showed a significantly lower frequency of tongue protrusion (Fig. 4A) and duration of twitching of the facial musculature (Fig. 4B), but did not show any statistical differences in these parameters when compared to saline-treated 4-month-old animals.

Discussion

The major findings of the present investigation were that: 1) reserpine-treated 4-month-old rats as well as older (20-month-old) rats developed orofacial movements characterized by increased tongue protrusion frequency and duration of twitching of the facial musculature; 2) reserpine induced an increase in striatal lipid peroxidation that was reversed by melatonin co-administration; 3) melatonin co-administration attenuated reserpine-induced orofacial movements; 4) melatonin administration attenuated spontaneous orofacial movements presented by older rats; and 5) continuous exposure to light increased the orofacial movements presented by saline- and by reserpine-treated 4-month-old rats.

Our results show that melatonin repeated treatment has similar effects on reserpine-induced orofacial movements and on age-induced orofacial movements. In this respect, the spontaneous orofacial movements presented by 20- to 24-month-old rats are phenomenologically identical to those induced by reserpine treatment in 4-month-old animals (Bergamo et al. 1997). Likewise, the frequently reported oral dyskinesia presented by elderly people (Wolfarth and Ossowska 1989) who had never undergone treatment with neuroleptics before is so similar to tardive dyskinesia that it is not possible to differentiate between these disorders on the basis of behavioral symptoms only (Gerlach 1985). Thus, the possibility is raised that related mechanisms are involved in these two phenomena. In terms of this conceptualization, it has been suggested that long-term neuroleptic treatment does not "cause" the emergence of orofacial movements; rather, such prolonged treatment may interact with some substrate of brain aging to result in premature emergence of an orofacial syndrome that can occur spontaneously in old age (Waddington 1990).

As stated earlier, increased formation of free radical byproducts seems to be related both to the aging process (Benzi and Moretti 1995) and to neuroleptic-induced tardive dyskinesia (Lohr 1991). Within this context, reserpine treatment may alter oxidative metabolism even more strongly than neuroleptics, since storage of newly synthesized dopamine is prevented and the dopamine is therefore continuously available for metabolism. In this respect, our results show that reserpine treatment increases striatal lipid peroxidation suggesting an enhancement of oxidative stress. This observation is in line with clinical studies showing increased levels of lipid peroxidation byproducts in blood or cerebrospinal fluid of tardive dyskinesia compared to non-tardive dyskinesia patients (Lohr et al. 1990).

This reserpine-induced oxidative stress was prevented by melatonin administration. In this way, the antioxidant properties of melatonin (Reiter 1998) could explain its attenuating effect on orofacial movements induced both by reserpine and age, and its protecting effect against reserpine-induced oxidative stress. This rationale supports the hypothesis that oxidative stress is a common factor underlying the pathophysiology of tardive dyskinesia and the aging process. Importantly, melatonin has been reported to rescue dopamine neurons from cell death in tissue culture models of oxidative stress (Iacoviti et al. 1997). Consistent with these observations, we have demonstrated that repeated monosialoganglioside administration attenuated reserpine-induced oral movements (Vital et al. 1997) as well as age-induced memory deficits (Silva et al. 1996) in rats. In this regard, Maulik et al. (1993) have reported that gangliosides can directly scavenge the oxygen free radicals both in vitro and in vivo. In further support of the hypothesis that the inhibitory effect of melatonin on reserpine-induced oral movements is related to its antioxidant properties, we have verified that reserpine repeated treatment induces an increase in the ratio of oxidized/reduced striatal glutathione, another index of the oxidative stress process (Toborek and Henning 1994; Bains and Shaw 1997), in rats, which was correlated with the increase in tongue protrusion frequency. Importantly, both these effects were attenuated by previous administration of vitamin E (a well known antioxidant agent) (C.C.S. Araújo et al., unpublished data). In addition, we have demonstrated that 3-nitropropionic acid previous and concomitant administration potentiates the increase in tongue protrusion induced by reserpine in rats (Calvente et al. 2002). In this regard, a large body of work has suggested that free radical generation may underlie the neurotoxic effects of succinate dehydrogenase inhibitors such as 3-nitropropionic acid and methylmalonic acid (Beal et al. 1995; Fighera et al. 1999).

Although it has been postulated that melatonin may attenuate orofacial movements due to its antioxidant properties, it is noteworthy that reserpine-induced tongue protrusions are mediated, at least in part, by residual endogenous dopamine. In this regard, although the destruction of presynaptic neurotransmitter vesicles produced by reserpine depletes dopamine, norepinephrine and serotonin (Oates 1996), experimental evidence suggests that dopamine is the monoamine critically related to reserpine-induced orofacial movements. Indeed, they are attenuated by acute neuroleptic administration (Neisewander et al. 1991b) and by nigrostriatal 6-hydroxydopamine lesions (besides being potentiated by amphetamine administration) (Neisewander et al. 1996). Interestingly, Sussman et al. (1997) demonstrated that reserpine-induced orofacial movements persisted despite

repletion of dopamine in the caudate-putamen at 84 days post-treatment. According to the authors, this finding suggests that dopamine may initiate reserpine-induced oral movements, but the persistent neuropathology may occur in a system efferent to the nigrostriatal pathway. In our results, the attenuation of reserpine-induced orofacial movements was more pronounced in the presence of melatonin, since the M+R-M group presented a decrease in both tongue protrusion frequency and duration of facial twitching as compared to the S+R-S group, while the M+R-S group presented a decrease in duration of facial twitching only. Thus, it can be reasoned that in addition to its antioxidant properties, a number of possible mechanisms involving the dopaminergic transmission can be invoked to explain the inhibitory effects of melatonin on oral movements. For example, in view of the fact that tardive dyskinesia is also associated with GABA deficiency (Fibiger and Lloyd 1984) a melatonin-GABA-dopamine interaction could also be considered, since melatonin enhances [^{3}H] GABA binding in rat brain (Coloma and Niles 1988) and striatal dopamine release is decreased by injection of GABA into the substantia nigra (Reid et al. 1988). In this regard, Tenn and Niles (1995) showed that the antidopaminergic effect of acute melatonin administration on the 6-hydroxydopamine model of striatal dopaminergic receptor supersensitivity was mediated by a GABAergic mechanism. In addition, in line with our results showing that long-term melatonin administration attenuates reserpine-induced orofacial movements, Raghavendra et al. (2001) observed that acute administration of melatonin also attenuates these movements.

In the present work, rats continuously exposed to light for 24 h presented a decreased level of serum melatonin. The suppression of melatonin secretion is therefore one of the most consistent biochemical effects of light exposure in mammals (Roberts 1995). Our results show that continuous exposure to light increases spontaneous orofacial movements as well as reserpine-induced orofacial movements suggesting that, independently of which mechanisms are involved in the ability of exogenous melatonin to attenuate reserpine- and age-induced orofacial movements, endogenous melatonin may play an important role in these phenomena. In this respect, melatonin is considered to be one of the most potent endogenous antioxidants (Reiter 1995) and its production deteriorates markedly with aging (Reiter 1995). In addition, both clinical and preclinical evidence provide further support to the notion that melatonin deficiency may be related to the pathophysiology of tardive dyskinesia. Indeed, while nocturnal plasma melatonin levels have been reported to be reduced in neuroleptic-treated schizophrenic patients (Ferrier et al. 1982), a significant association between pineal calcification and the presence of tardive dyskinesia was found (Sandyk and Kay 1991). Furthermore, reduction in melatonin secretion has been suggested to be related to tardive dyskinesia associated with depression in bipolar patients (Sandyk 1990). Finally, the incidence and severity of haloperidol-induced orofacial movements have been shown to be increased in pinealectomized rats (Sandyk and Fisher 1989).

In conclusion, the results from the present study are consistent with the hypothesis that endogenous melatonin may be involved in reserpine- and age-induced orofacial movements. Moreover, despite the exact mechanism underlying the antidyskinetic properties of melatonin, our data related to long-term treatment, taken together with those of Raghavendra et al. (2001) related to acute administration, open the possibility that melatonin could be useful in the prevention and treatment of orofacial movements.

Acknowledgements This research was supported by fellowships from FAPESP (Procs. 1995/9462-7 and 98/13448-8), CNPq (Proc. 522975/95), CAPES, AFIP and FADA. The authors would like to thank Mrs. Teotila R.R. Amaral and Mr. Cleomar S. Ferreira for capable assistance.

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