treatment schedule used was comparable to that of this study, since lead was administered to the animals as a 0.2% lead acetate drinking solution. In contrast, no difference was found when the exposure occurred after weaning[8].

The observations of this study lend further support to the idea that developing serotonergic neurons are also affected by chronic lead exposure.

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Melatonin modulates apomorphine-induced rotational behaviour

S. Burton, S. Daya* and B. Potgieter

*School of Pharmaceutical Sciences, and * Department of Biochemistry, Rhodes University, Grahamstown 6140 (South Africa)*
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Summary. Prior melatonin administration (1 and 10 mg/kg b.wt) causes a significant reduction in apomorphine (1 mg/kg b.wt) induced rotational behaviour in both 6-hydroxydopamine and quinolinic acid lesioned rats.
Key words. Melatonin; apomorphine; 6-hydroxydopamine; quinolinic acid; rotational behaviour.

The regulatory function of the pineal gland, ideally situated anatomically to integrate and compare information from both extra- and intra-cranial sources, appears to be mediated through release of the principal pineal hormone, melatonin (MEL)[1]. Apart from the neuroendocrine role of MEL[2], animal and human studies have implicated MEL as an important modulator of behaviour.

Conflicting reports on the effect of MEL in Parkinson's disease – a movement disorder characterized by striatal DA deficiency exist. Anton Tay et al. report improvement of symptoms on administration of 1.2 g/day of MEL to Parkinsonian patients[6] while Shaw et al. report that doses of MEL up to 1 g/day did not alter the Parkinsonian syndrome[7]. In schizophrenia – a disorder possibly associated with DA hyperactivity, MEL metabolism appears to be altered[8] and reduced midnight levels of MEL coupled with raised cortisol levels have been reported[9]. The role of the pineal gland and MEL's antidyskinetic activity has begun to be recognised in neuroleptic-induced tardive dyskinesia, a disorder associated with a supersensitivity of DA receptors[10]. MEL has also been demonstrated to cause 'psychomotor retardation' when administered to patients with Huntington's chorea, a movement disorder characterized by hyperkinetic choreiform movements, which is primarily treated with DA antagonists[11].

High affinity binding sites for ^{125}Iodo-MEL have been identified in the striatum, as well as the hippocampus, hypothalamus, cortex and amygdala of both the male

and female rat[3]. The hypothalamic binding sites appear to correlate with MEL's neuroendocrinal activity but "the role of striatal ^{125}Iodo-MEL binding sites is still an enigma"[24]. MEL has been shown to regulate forebrain dopaminergic functions in the rat. Bradbury and coworkers demonstrated inhibition of behavioural locomotor activity following intranigral injection of MEL[5]. This effect was accompanied by reduced dopamine (DA) 'function' in the striatal and limbic systems as determined by increased DA content and a reduced DOPAC/DA ratio, suggesting a reduction in the release of DA in these brain areas. These effects were partially reversed by the D_2 selective antagonist sulpiride. It has been suggested that DA autoreceptors may be involved in the inhibition of DA functions by MEL[5]. The results of all these studies led to the proposal that MEL has a modulatory influence on central nervous system dopaminergic activity, particularly in the nigrostriatal system and that the pineal gland and MEL may be involved in the pathophysiology of DA-related movement disorders[5,12].

The quantification of apomorphine (APO)-induced turning behaviour in rats with a unilateral lesion of the nigrostriatal system is a useful model for studying several aspects of dopaminergic function in the central nervous system. Two variations of this model are 1) an animal model of Parkinson's disease – where rats are lesioned in the substantia nigra with 6-hydroxydopamine (6-OHDA) which produces a supersensitive dopamine (DA) receptor model[13], and 2) an animal model of Huntington's chorea – where rats are lesioned in the corpus striatum with quinolinic acid (QA) to produce a normosensitive DA receptor model[14]. Both models were used in this study to investigate whether MEL may have a role in the modulation of central dopaminergic function.

Materials and methods

Male Wistar rats weighing 140–160 g at the time of the stereotaxic injections were used in all experiments. At the time of subsequent rotation experiments, the rats had acquired a mass of 225–275 g. The animals were housed 4 in each cage prior to stereotaxic procedures and individually thereafter. They were maintained in a temperature-controlled environment (21–23 °C) on a regulated 12 h light (06.00–18.00): 12 h dark light cycle with food and water ad libitum. Rats were lesioned under sodium pentobarbital (Sagatal – May and Baker – 42 mg/kg b.wt) induced anaesthesia. The rat skull was orientated according to the Konig and Klippel stereotaxic atlas[15]. A Hamilton syringe with a cannula of 0.3-mm diameter was used to inject 8 µg of 6-OHDA-HCl (calculated as base, SIGMA) in 4 µl of ice-cold saline (with 0,2% ascorbic acid as antioxidant) into the pars compacta zone of the substantia nigra or two stereotaxic injections of 1 µl of 150 nanomolar solution of QA (SIGMA) prepared in ice-cold saline into the left striatum (coordinates: 1.0 mm anterior from bregma, 2.3 mm lateral and 4.5 mm vertical, and 3.8 mm lateral at the bregma level and 4.5 mm vertical). Injections were administered at a rate of 1 µl per minute and the cannula was left in situ for a further 3 min following drug injection to allow for passive diffusion away from the cannula tip and to minimise spread into the injection tract. Animals were kept warm until recovery from anaesthesia. Rats used as controls were subjected to the surgical procedures outlined above. However stereotaxic injections into the brain regions in both cases were free of the drugs 6-OHDA and QA and comprised only the vehicle – saline and ascorbic acid.

Rotational behaviour was observed in a flat-bottomed circular plastic bowl (diameter: 800 mm). An observer recorded the number of complete 360° turns, either wide or tight head to tail pivotal turns made by the rats in a pre-specified time period. All animals were placed in the test environment for a 30-min habituation period prior to drug administration and observation of turning behaviour. The total number of complete 360° turns was recorded manually with contralateral turns being recorded as negative and ipsilateral turns as positive. Circling was expressed as net total turns which were obtained as the algebraic sum of the contralateral and ipsilateral turns. In order to select the successfully lesioned animals, rats were challenged with low doses of APO (0.5 mg/kg b.wt) two weeks after lesioning. Only rats which demonstrated vigorous (at least 3 turns per minute) and reliable turning were selected for further experimentation. Successfully 6-OHDA-lesioned rats showed contralateral rotation and successfully QA-lesioned rats showed ipsilateral turning. The effect of i.p. administered MEL (1 and 10 mg/kg b.wt), 5 min prior to challenge with APO 1 mg/kg b.wt, on APO-induced turning behaviour was observed and recorded in groups of 5 and 7, 6-OHDA and QA-lesioned rats, respectively. Rotation was recorded as the net total turns during the 20-min period beginning 5 min post APO administration. All rats were tested for response to APO 1 mg/kg, 21 days post lesioning and the effect of MEL 1 and 10 mg/kg on this response was determined in a crossover study at 28 and 42 days after lesioning. All daytime experiments were carried out between 14.00 and 16.00 h with an illumination level immediately above the test environment of approximately 1800 lux, conditions under which endogenous MEL levels are negligible[16]. Control experiments included: 1) the effect of MEL on lesioned rats in the absence of APO; 2) the effect of APO 1 mg/kg b.wt on sham-operated rats; 3) the effect of APO 1 mg/kg b.wt on unoperated rats.

In order to investigate a possible effect of endogenous MEL on central dopaminergic function, the effects of APO (1 mg/kg b.wt) administration, 5 h after the onset of darkness (23.00), on rotational behaviour in the 6-OHDA lesioned rats was studied. The results of such observations were compared to the data obtained from lesioned rats which had been exposed to white light (1800 lux) for a 20-min period 5 h after the onset of the

dark period to physiologically pinealectomize them[17,18], rehabituated to the dark for 10 min and then challenged with APO (1 mg/kg b.wt), and lesioned rats challenged with APO (1 mg/kg b.wt) during the light phase (at 14.00). Rotational behaviour was observed in all experiments by an independent observer denied knowledge of drug treatment. APO (SIGMA) was dissolved in saline and 1 mg/ml ascorbic acid was included as anti-oxidant. APO was always administered s.c. in the flank. MEL (SIGMA) injections were prepared in the following vehicle and were always administered by the i.p. route (Benzyl alcohol 200 µl, anhydrous citric acid 25 mg, TWEEN 80 1 ml, water for injection to 10 ml). Means and SEM were calculated and data was analysed using analysis of variance with Scheffe's post hoc test for multiple comparisons. A value of $p < 0.05$ was considered as statistically significant.

Results

At the doses tested (1 and 10 mg/kg b.wt) i.p. administered MEL significantly inhibited turning behaviour induced by APO 1 mg/kg BM administration. MEL significantly reduced the net total turns to a level that was not significantly different from that seen in sham lesioned and unoperated animals in 6-OHDA lesioned rats (fig. 1). In QA lesioned rats, although the turning response to APO was reduced, it still remained significantly greater than the response seen in controls (fig. 2). In both models MEL administration alone failed to have any significant effect on the behaviour of lesioned animals. At the doses used this effect of MEL was not shown to be significantly dose dependent, as both doses of MEL reduced turning behaviour to the same degree in both models. Night-time experiments carried out in the dark at 23.00 showed a circadian variation in APO-induced activity. When the endogenous levels of MEL were high

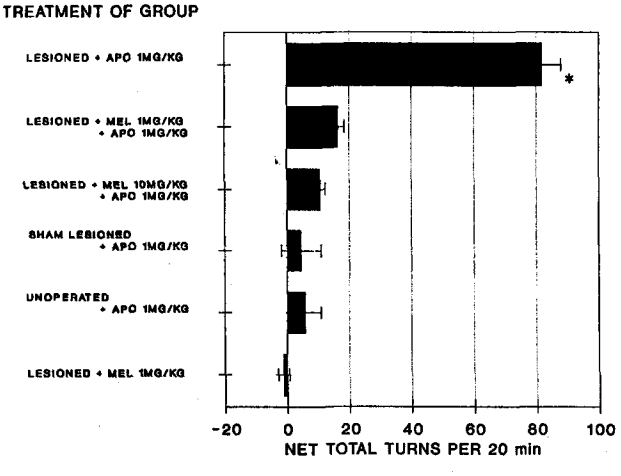

Figure 1. Turning behaviour evidenced by various groups of rats in the 6-OHDA-lesioned model. All bars represent the mean ± SEM of N = 5 rats. The symbol * indicates a statistically significant difference with respect to all other treatment groups ($p < 0.05$). Contralateral turns are represented as positive turns and ipsilateral turns as negative turns.

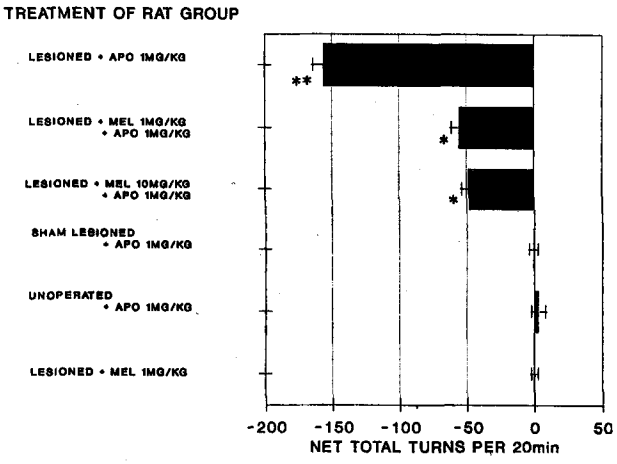

Figure 2. Turning behaviour evidenced by various groups of rats in the QA-lesioned model. All bars represent the mean ± SEM of N = 7 rats. The symbol ** indicates a statistically significant difference with respect to all other treatment groups ($p < 0.05$), while the two treatment groups indicated by the symbol * are not statistically different to each other but are statistically different to all other treatment groups ($p < 0.05$). Contralateral turns are represented as positive turns and ipsilateral turns as negative turns.

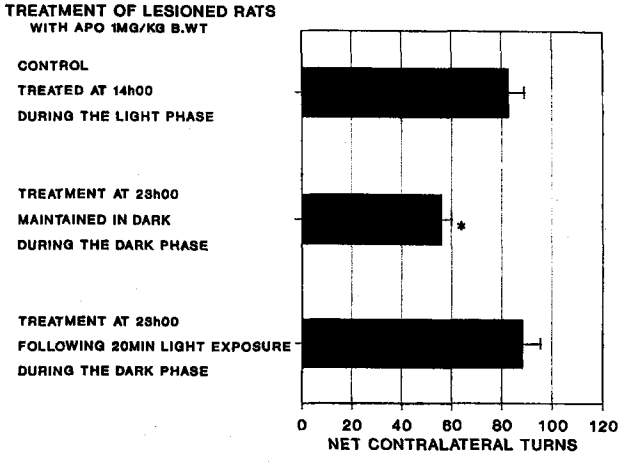

Figure 3. The effect of exposure to light on the APO (1 mg/kg b.wt) induced rotational response in 6-OHDA-lesioned rats, during the middle of the dark phase. All bars represent the mean ± SEM of N = 5 rats. The symbol * represents a statistical difference with respect to other treatment groups ($p < 0.05$). Contralateral turns are represented as positive turns and ipsilateral turns as negative turns.

(23.00)[16] APO administration gave significantly lower activity levels than when administered during the day (fig. 3). Functional pinealectomy by exposure to bright light at 23.00[17,18] returned the activity to daytime levels (fig. 3).

Discussion

Results indicate that MEL has a significant effect on striatal dopaminergic function, of which the APO-induced turning behaviour in both these models acts as an index. Early speculation following results obtained with the 6-OHDA model suggested that MEL may normalize

or compensate for the sensitivity of supersensitive DA receptors in the lesioned striatum[13]. However in this study MEL antagonised turning behaviour induced by APO activity in QA-treated rats in which the DA receptors are reputed to be normosensitive. This suggests that MEL does not act by normalizing receptor sensitivity. Although the nigrostriatal dopaminergic system appears to be the primary system involved in the APO-induced rotational response, the modifying role of other neurotransmitter systems and other cerebral regions cannot be ignored. Treatments which inhibit or damage other neurotransmitter pathways, for example noradrenergic, serotonergic, cholinergic and GABA-ergic, modify the rotatory effects of dopaminergic agonists[19]. These studies do not rule out the possibility that MEL may be acting via one of the alternative pathways to modify the APO-induced rotational response. The results of the present study do however show that MEL modifies the behavioral response of the central nervous system to the DA agonist APO, supporting the concept that MEL may have a modulatory role on dopaminergic transmission in the central nervous system. The QA-lesioned rat has been proposed as a model for Huntington's chorea[20], and it is significant to note that MEL inhibited APO-induced turning in these rats. The 6-OHDA model is an accepted animal model of Parkinson's disease and is useful to screen anti-Parkinsonian drugs[21]. Anti-Parkinsonian agents are characterized by their ability to induce rotation in lesioned rats. MEL's ability to inhibit the induction of APO-induced turning suggests, therefore, that MEL probably is not beneficial in the treatment of Parkinson's disease and may even worsen the symptoms. Further clinical studies will be required to confirm this animal work. MEL may however be useful in the treatment of other disorders such as schizophrenia, Huntington's chorea and tardive dyskinesias, all of which are associated with DA receptor supersensitivity or DA overactivity.

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