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Short communication

Effects of a seven-day continuous infusion of L-DOPA on daily rhythms in the rat

Nicolas Simon, Jacques Mouchet, Bernard Bruguerolle *

Laboratoire de Pharmacologie Médicale (Développement et Pathologie du Mouvement, UMR-FRE), Faculté de Médecine de Marseille, Université de la Méditerranée, 27, Bd J. Moulin F-13385, Marseille Cedex 5, France

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Abstract

The present study was conducted to evaluate the effect of L-3,4-dihydroxyphenylalanine (L-DOPA) on the daily rhythms of temperature, heart rate and locomotor activity in rats that received a 7-day continuous infusion. Our results indicate that L-DOPA does not induce a loss of the daily rhythmicity of temperature, heart rate and locomotor activity but modifies the main parameters of these rhythms, e.g. it increased the MESOR (midline estimating statistic of rhythm) of temperature and heart rate and increased the amplitude of temperature but decreased the amplitude of heart rate. Taking into account these results obtained after constant rate delivery, we now plan to investigate the effects of DOPA therapy by changing the time of its administration.

Keywords: L-DOPA methylester; Parkinson's disease; Daily rhythm; Chronopharmacology; Radiotelemetry; Infusion constant rate

1. Introduction

We have previously reported an animal model of Parkinson's disease consisting of a 6-hydroxydopamine double striatal bilateral, e.g. anterior and posterior, lesion in rats (Ben et al., 1999). The lesion induces a loss of striatal dopaminergic terminals and provides a good tool to meet our experimental need for future chronopharmacological studies on anti-Parkinsonian drugs. In such a model, biological rhythms, e.g. temperature, heart rate and locomotor activity, continuously registered by radiotelemetry, were shown to be differently affected according to time; temporary loss of daily periodicity for heart rate, decrease of the MESOR (midline estimating statistic of rhythm) and advance of the acrophase of the three rhythms. Such findings supply a basis for the future study of chronopharmacological aspects of anti-Parkinsonian drugs (Bruguerolle, 1998), and we plan to demonstrate that such induced perturbations may be corrected by changing the time of day of administration of L-DOPA and/or other dopamine agonists. However, we need to know the effects of dopamine agonists on the daily rhythms in control animals before we can evaluate their effects in our model of Parkinson's disease. Thus, the present study was conducted to evaluate the effect of L-3,4-dihydroxyphenylalanine (L-DOPA) on the daily rhythms of temperature, heart rate and locomotor activity continuously registered by radiotelemetry, after continuous delivery of the drug for 7 days.

2. Material and methods

2.1. Animals

For a minimum of 3 weeks before use, seven Wistar AF IOPS adult male rats from IFFA-CREDO (St. Germain-sur-l'Arbresle, France), mean weight, 275 g and 10 weeks old, were housed in individual transparent polypropylene cages under controlled environmental conditions, i.e. relative humidity (50%–55%), temperature (24 ± 1°C), and synchronisation by a light–dark cycle (12:12; light from 06:00 to 18:00 h; dark from 18:00 to 06:00 h). Food and

* Corresponding author. Tel.: +33-4-91-83-44-56; fax: +33-4-91-25-65-26.

E-mail address: bernard.bruguerolle@medecine.univ-mrs.fr (B. Bruguerolle).

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water were available ''ad libitum'', changes occurring one time per week on an irregular schedule. The experiments were conducted in accordance with internationally accepted principles concerning the care and use of laboratory animals (NIH Publication no. 85-23, 1985) after acceptance by our animal experimentation ethics committee (Commission Consultative d'Ethique Animale, Centre de Formation et de Recherches Experimentales Medico-Chirurgicales, Faculte de Medecine de Marseille, France).

2.2. Experimental procedures

Temperature, heart rate and locomotor activity were measured by means of radiotelemetry. The surgical implantation of the transmitters (model TA11-CTA-F40®, Data Sciences, St. Paul, MN, USA) was performed under equitesine (4 ml/kg i.p. of a 100 ml mixture: chloral hydrate, 4.6 g; magnesium sulfate, 2.12 g; pentobarbital, 0.960 g; propylene glycol, 42.8 ml; alcohol, 10.8 ml in 30.4 ml of saline) general anaesthesia, which lasted 45 min. Just before implantation, calibration values of each transmitter were entered into the Dataquest III® data acquisition system (Data Sciences). Transmitters were implanted as described in detail by Kramer et al. (1993). Briefly, under complete anaesthesia, a 2-cm incision was made in the peritoneum and the telemetric sender was implanted into the abdominal cavity and sutured to the abdominal wall. Electrocardiographic (ECG) leads were extended subcutaneously to the right axilla and to the left lower rib area, and sutured to muscle tissue there. After closure, animals were monitored until they recovered from anaesthesia, and then were returned to their home cage. Signals from the transmitters were received by an antenna mounted in a receiver board (model CTR86®, Data Sciences) placed under the animal's cage. Data of temperature (°C), heart rate (beats/min, i.e. bpm) and locomotor activity (counts) were continuously monitored, collected every 10 min over a 28-day period and processed by a PC with a specialized recording and analysis system (Dataquest III®, Data Sciences).

2.3. Protocol of the study

After a recovery period of 2 weeks from surgical implantation of the telemetric devices and/or anaesthesia, the study was divided into a first 7-day control period (C) for baseline measurements of daily temperature, heart rate and locomotor activity rhythms. This period was only characterized by daily handling and weighing of animals (09:00–09:10 h). At the end of this period, rats were implanted subcutaneously under ether anaesthesia with mini-osmotic pumps (Alzet®, 2 ML1) filled with saline containing ascorbic acid (0.1%) ($n = 3$, controls) or L-DOPA methyl ester in ascorbic acid ($n = 4$, treated, T) at a concentration calculated to deliver 100 mg/kg/day at a continuous rate (10.0 μl/h) for 7 days; a second 7-day registration period was thus observed (T). At the end of this period, the mini-osmotic pumps were removed under light ether anaesthesia. Finally, a 7-day recovery period was registered.

2.4. Telemetric data analysis

Temperature, heart rate and locomotor activity were continuously monitored and plotted every 10 min. Data were analyzed by two methods using Dataquest III® software. First, in order to determine the dominant period of the three rhythms for the control period and the following 4 weeks, a power spectrum analysis (Fourier Transform) was applied to 30 min average data intervals. Power spectrum analysis allowed examination of the spectral content of the raw, telemetered data series. The computed power spectrum showed peaks at the frequency corresponding to the period of the variation in the data series. Then, in order to assess the daily variations of temperature, heart rate and locomotor activity, single cosinor analysis was applied to individual data for the control period and the following 4 weeks and a rhythm was considered to be significantly detected when $P < 0.001$ (De Prins and Hecquet, 1992). The circadian rhythm characteristics of temperature, heart rate and locomotor activity, i.e. MESOR (corresponding to the mean level which is equal to the 24 h average), amplitude (half of the peak-to-trough difference of the fitted cosine function) and acrophase (the crest time of rhythm given in degrees, where 360° corresponded to a 24-h cycle and the starting time of 00.00 h was assessed as 0°) were estimated by the linear method of least squares (12) and expressed as means ± S.E.M. For each rat, the body weight was measured and expressed as means ± S.E.M. for each group. A two-way (treatment: controls vs. L-DOPA and period of the protocol: control, treatment and recovery periods) analysis of variance (ANOVA) was used for statistical analysis (Statview II® program). Then, the comparisons between treatments and for each group between the different periods were done by a one-way ANOVA, followed, if a significant difference was found, by a Fisher's PLSD (protected last significant difference) test for multiple comparisons.

3. Results

3.1. Weight

The weight gain with time was not statistically different between the two groups of rats throughout the experiment.

3.2. Telemetric data

Fig. 1 shows an example of raw data for the three studied physiological parameters in a rat of each group

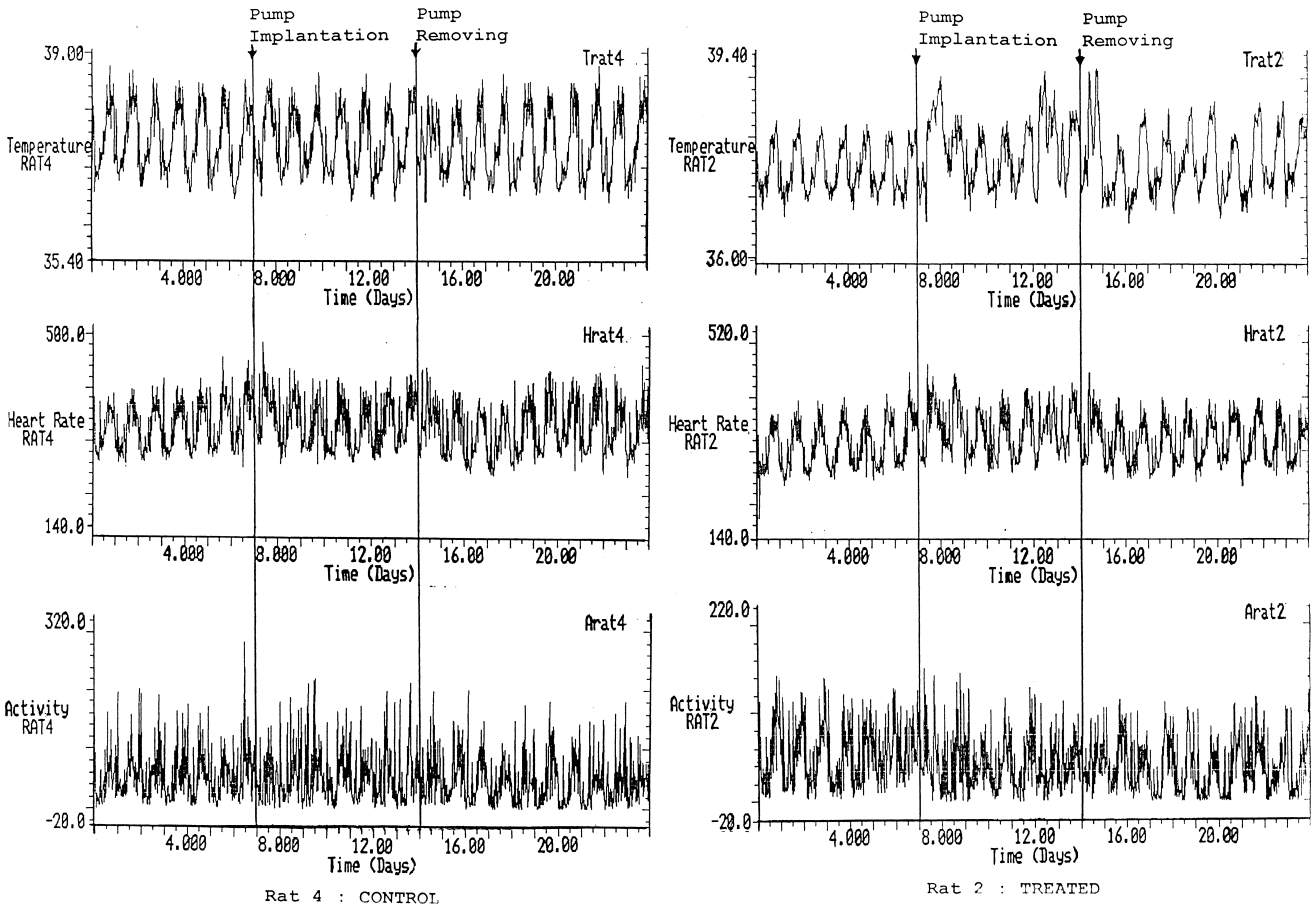

Fig. 1. Raw values (continuously monitored) for body temperature, heart rate and locomotor activity during the 3 weeks of the study in a control (rat no. 4) and a L-DOPA-treated rat (rat no. 2).

(control and L-DOPA treated) over the entire experimental period.

3.2.1. Induced modifications of periodicity

As indicated by Fourier analysis (not shown), the periodicity of the three rhythms registered, e.g. temperature, heart rate and locomotor activity, was not significantly different during the three experimental periods (control, treatment, recovery).

3.2.2. Induced modifications of the rhythm characteristics: statistical analysis of cosinor parameters

The continuous infusion of L-DOPA did not perturb the daily periodicity of temperature, heart rate and locomotor activity, but modified some of the characteristics of these rhythms as indicated in Table 1. There was an increase in the MESOR of temperature and heart rate, an increase in the amplitude of temperature and a decrease in the amplitude of heart rate. One can notice that the increase in the amplitude of temperature and the decrease in the amplitude of heart rate observed in treated rats during the treatment period were also observed and amplified during the recovery period. These changes were not significant in the controls during the recovery period, thus, indicating a prolonged effect of L-DOPA. The characteristics of the daily locomotor activity rhythm were not significantly affected.

4. Discussion

Our data document that the daily rhythms of temperature, heart rate and locomotor activity were not suppressed, but were differently affected by a continuous infusion of L-DOPA: there was an increase in the MESOR of temperature and heart rate, an increase in the amplitude of temperature and a decrease in the amplitude of heart rate. Since a loss of circadian rhythmicity may only be demonstrated under constant light or dark conditions, the masking effect of light/dark periodicity, which may be observed under the light/dark 12:12 h conditions of the present study, does not allow us to affirm a lack of circadian rhythmicity loss. Nevertheless, under the light/dark 12:12 h conditions of our study, L-DOPA did not affect the observed daily rhythms.

The use of a continuous constant rate of delivery of L-DOPA by osmotic pump in the present work aimed to

Table 1
Characteristics (MESOR, amplitude and acrophase) of the circadian rhythm of temperature, heart rate and locomotor activity in control and L-DOPA-treated rats according to the experimental period (means ± S.E.M.)

	Temperature					
	MESOR (°C)		Amplitude (°C)		Acrophase (°)	
	Saline	L-DOPA	Saline	L-DOPA	Saline	L-DOPA
Control	37.45 ± 0.04	37.57 ± 0.02	0.602 ± 0.02	0.498 ± 0.015	−1.38 ± 2.92	−5.94 ± 1.72
Treatment	37.44 ± 0.03	37.70 ± 0.04[a,b]	0.590 ± 0.02	0.595 ± 0.031[a]	−354.82 ± 1.99	−2.98 ± 4.05
Recovery	37.43 ± 0.03	37.53 ± 0.04	0.620 ± 0.02	0.623 ± 0.020[c]	−321.08 ± 3.86	−357.69 ± 3.29
ANOVA	$P = 0.876$	$P = 0.001$	$P = 0.666$	$P = 0.0006$	$P = 0.30$	$P = 0.183$
	Heart Rate					
	MESOR (b/m)		Amplitude (b/m)		Acrophase (°)	
	Saline	L-DOPA	Saline	L-DOPA	Saline	L-DOPA
Control	338.03 ± 3.93	326.09 ± 4.06	48.66 ± 1.30	52.02 ± 1.32	−308.40 ± 5.17	−355.45 ± 1.62
Treatment	351.03 ± 4.71	342.13 ± 3.14[a,b]	43.86 ± 2.45	48.18 ± 2.34	−347.90 ± 5.06	−349.41 ± 3.19
Recovery	336.47 ± 4.65	326.58 ± 2.70	49.15 ± 3.18	44.87 ± 1.44[c]	−351.08 ± 4.69	−350.77 ± 3.94
ANOVA	$P = 0.06$	$P = 0.001$	$P = 0.250$	$P = 0.020$	$P = 0.536$	$P = 0.367$
	Locomotor Activity					
	MESOR (counts)		Amplitude (counts)		Acrophase (°)	
	Saline	L-DOPA	Saline	L-DOPA	Saline	L-DOPA
Control	41.39 ± 2.88	41.18 ± 3.11	25.64 ± 1.20	28.73 ± 2.10	−7.83 ± 2.87	−19.12 ± 2.71
Treatment	46.42 ± 3.22	33.60 ± 3.36	28.37 ± 1.92	26.39 ± 2.74	−4.83 ± 4.30	−336.23 ± 13.32
Recovery	44.76 ± 3.51	37.55 ± 3.63	31.59 ± 2.24	29.16 ± 3.16	−5.24 ± 2.53	−11.80 ± 2.88
ANOVA	$P = 0.520$	$P = 0.303$	$P = 0.075$	$P = 0.751$	$P = 0.783$	$P = 0.146$

Statistical analysis was done by a two-way [treatment (control vs. L-DOPA-treated rats) and period of the protocol (control, treatment and recovery)] ANOVA. Then, the comparisons between treatments, and for each group between the different periods were done by a one-way ANOVA, followed, if a significant difference was found, by a Fisher's PLSD test for multiple comparisons.

[a] $P < 0.05$ treatment vs. control period.

[b] $P < 0.05$ treatment vs. recovery.

[c] $P < 0.05$ recovery vs. control period.

eliminate a possible influence of daily handling and injection stress and to ''control'' the influence of the hour of administration of L-DOPA, i.e. a possible chronopharmacological effect, in order to observe the proper effects of the drug on temperature, heart rate and locomotor activity daily rhythms. One could argue that the observed modifications of the daily rhythms may be partly due to the use of anaesthetics for pump implantation. However, given the lack of effect in controls, anaesthesia does not play a role in the observed perturbations of the daily rhythms. This agrees with previously published data (Prudian et al., 1997) on the lack of effect of ether anaesthesia on daily rhythms. It has been documented that ketamine and ether affected daily rhythms differently, with ketamine inducing major alterations, particularly on the daily heart rate rhythm. The present work showed a statistically significant increase of the amplitude of temperature in treated rats and during the recovery period, but not in respective controls, indicating a prolonged effect despite removal of the pumps. The same effect was observed for the decrease of the amplitude of heart rate. This may be related to the well-known increase in 3-*O*-methyl dopa, a major metabolite of L-DOPA, after repeated administration of L-DOPA.

The present study indicates that daily rhythms are modified after continuous infusion of L-DOPA at a constant rate, demonstrating the effects of dopamine formed after L-DOPA administration. The effects of dopamine on temperature, heart rate and motor activity are well known but the effects on the daily rhythms of these physiological parameters have not been studied. Fluctuations in dopamine metabolism, overnight dopamine accumulation or cyclic receptor down-regulation, as demonstrated by Lemmer and Berger (1978), Kafka et al. (1983) and Naber et al. (1981), may explain the effects of exogenous dopamine on the dopaminergic central nervous structures possibly involved in the control of the circadian system (Lange et al., 1995). Thus, the effects of L-DOPA are not surprising, but it is particularly interesting to notice that despite continuous infusion for 7 days at a constant rate, rhythms such as temperature, heart rate and locomotor activity are not suppressed and are relatively smoothly modified, even if light/dark masking effects may be partly responsible for

this effect. Taking into account these results obtained with constant rate delivery, we now plan to investigate (1) the effects of such a continuous infusion in our animal model of Parkinson's disease and (2) the effects of varying the time of delivery, i.e. a chronopharmacological approach of dopa therapy by changing the time of day of administration of L-DOPA.

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