A functional subdivision of the circadian clock is revealed by differential effects of melatonin administration

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Abstract

The biological clock of the suprachiasmatic nuclei drives numerous physiological and behavioural circadian rhythms. In this study, we addressed the question as to whether different components of the clock may control separately various circadian functions. Using the rat transpineal microdialysis tool, we analysed the effect of clock perturbation by exogenous melatonin injection on two hormonal clock outputs: pineal melatonin and adrenal corticosterone secretions. As already reported, a single melatonin injection at the light/dark transition induces a marked increase in the endogenous pineal melatonin peak for the two following days. In the same animals, by contrast, the amplitude of the corticosterone rhythm was not altered following melatonin injection. These data show that the melatonin injection does not display an overall effect on the circadian clock, but rather influences a subpopulation of melatonin-sensitive neurons involved, among other functions, in the circadian control of the pineal pathway.

Keywords: Melatonin; Corticosterone; Suprachiasmatic nuclei; Circadian

In mammals, the central biological clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus, is known to control daily rhythms in behaviour as well as in physiological and hormonal parameters [3], of which the rhythm of melatonin synthesis by the pineal gland is one of the best examples [15,18]. One unresolved question concerning the extensive SCN control of numerous daily rhythms is whether it involves the entire SCN or several subsets of SCN cells. To address this question, we used the well-known feedback effect of melatonin on SCN rhythmicity. When applied at the light/dark transition, endogenous melatonin can entrain circadian rhythms in vivo [19,21] or in vitro [13]. Additionally, using the transpineal microdialysis technique, we have shown that application of exogenous melatonin induces a long-term increase in the amplitude of endogenous secretion of melatonin. This effect is the result of a direct action of melatonin on the SCN, and was observed only when melatonin is applied at the light/dark transition [2].

To test whether the clock controls separately its various outputs, we capitalized on this effect of exogenous melatonin. If melatonin induces a global increase in clock oscillations, we should observe an increase in the amplitude of other clock-controlled rhythms. The daily rhythm of corticosterone secretion is a suitable candidate because this hormone displays a typical circadian rhythm [14] in which, as for melatonin, the pivotal role of the autonomic nervous system has been demonstrated [4]. Using our recently developed microdialysis technique allowing simultaneous assay of both melatonin and corticosterone secretion in the same free-moving animals over several days [16], we decided to study the effect of a single melatonin injection, at the light/dark transition, on the daily rhythms of both melatonin and corticosterone for three consecutive days in the same animals.

Male Wistar rats (n = 12; 250–300 g) were purchased from Dépré (Douchard, France). The animals were housed in a temperature-controlled room (21 ± 2 °C) and maintained on a 12-h light:12-h dark lighting regimen (LD 12:12; lights off from 19:00 until 07:00 h) with dark onset corresponding to Zeitgeber time ZT12. Water and food were available ad libitum. All experiments with animals were performed in accordance with “Principles of laboratory animal care” (NIH pub. No. 86-23, revised 1985) as well as with the French national laws.

The animals were anaesthetised with tiletamine/zolazepam (20 mg/kg body weight, i.p.) and xylazine (15 mg/kg body weight, i.p.). After surgery, animals were allowed to recover for one week in individual cages. Implantation of the micro-
dialysis probe in the pineal gland was performed as previously described [1,7]. The dialysis membrane was saponified cellulose ester (0.22 mm inside diameter, 0.27 mm outside diameter, 10,000 molecular mass cut off). During the experiment, the probe inlet was connected by polyethylene tubing to a microinjection pump (PhD 2000, Harvard, Les Ulis, France) via a fluid swivel (375/22, Instech Laboratories, Plymouth Meeting, PA, USA). The swivel was attached to a counterbalance beam allowing the animal to move freely. The probe was perfused with Ringer’s solution at a flow rate of 3 μl/min. The outlet connection of the probe consisted of polyethylene tubing (0.3 mm inside diameter, 0.7 mm outside diameter) connected to a 1.5 ml polypropylene microvial. Another polyethylene tube catheter was subcutaneously implanted in each animal to inject melatonin or vehicle without handling the animals.

Rats were divided into two groups: vehicle-injected (Group I) and melatonin-injected (Group II). Melatonin (Sigma, Saint Quentin Fallavier, France; 1 mg/kg b.w. dissolved in 5% ethanol/Ringer) or vehicle (5% ethanol in Ringer) were injected subcutaneously through the previously implanted catheter (ZT12 being the only time at which melatonin administration had an effect on the amplitude of the endogenous melatonin rhythm [2]). Dialysates were collected hourly from ZT0 to ZT24 the day before (Day 1) and two days after (Days 3 and 4) melatonin or vehicle administration (on Day 2). Sampled dialysates were stored at −20 °C until assayed by radioimmunoassay (RIA). At the end of the experiment, the rats were decapitated and the brain dissected with pineal gland. The brain was frozen and cryostat sections of the brain/pineal were mounted for microscope observation to control for probe location.

Melatonin concentration in dialysates was determined in duplicates of 25 μl samples by RIA using a specific rabbit anti-serum (R19540 final dilution of 1/90,000) provided by INRA (Nouzilly, France) and [125I]-2-iodomelatonin. The limit of sensitivity of the assay was 0.5 pg/tube. The direct melatonin concentration in dialysates was determined in duplicates of 25 μl samples by RIA using a specific rabbit antiserum [1]. Corticosterone concentration in dialysates was determined in duplicates of 25 μl samples by RIA using a commercially available [125I]-corticosterone RIA kit (ICN Biomedical Division, Carson, CA, USA). The limit of sensitivity of the assay was 25 pg/ml. The method was previously validated for dialysates [16].

Data from all experiments are expressed as mean ± S.E.M. of correctly implanted animals (Group I, n = 4 out of 6; Group II, n = 5 out of 6). The highest value of each hormonal peak observed during the control Day 1 was taken as 100 (in arbitrary units), and all other values observed during the following days were expressed relative to this reference. For each group, raw data were analysed using a two-way MANOVA considering the day (three levels: Days 1, 3 and 4) and the time of day (16 or 25 levels depending on the hormone) as repeated measures within subject factors. If F values were appropriate, MANOVA analyses were followed by a Student–Newman–Keuls post hoc test. The level of statistical significance was set at 0.05 throughout.

As previously demonstrated, successive melatonin profiles measured in the same animal were highly reproducible over several days. No effect of vehicle injection was detected (Fig. 1A).

The duration of the control melatonin peak obtained by nonlinear regression [2] was 5.81 ± 0.11 h. By contrast, melatonin injection on Day 2 induced a sustained increase in amplitude of melatonin secretion on Days 3 and 4 compared to that measured on Day 1 in the melatonin-injected Group II. MANOVA revealed a significant increase [F(2, 8) = 22.42, p < 0.001] of 100–130% in the amplitude observed on Day 3 (p < 0.01) as well as on Day 4 (p < 0.01) compared to Day 1 (Fig. 2A).

The corticosterone secretion profile obtained by transpineal microdialysis was also highly reproducible for several days in the vehicle-injected Group I (Fig. 1B). A significant rhythmic secretion was detected [F(23, 69) = 7.28, p < 0.001] with maximal values at the light/dark transition, without significant effects of vehicle injection given at ZT12 on Day 2. The corticosterone concentration was minimal during the first part of the light period, then started to increase at about ZT8 to reach maximal values from the light/dark transition until the middle of the dark period. Duration of the corticosterone peak obtained by non-linear regression was 10.92 ± 0.31 h. Melatonin injection had no effect on the corticosterone secretion profile in melatonin-injected Group II (Fig. 2B). Statistical analysis did not reveal
system controlling adrenal corticotrophin sensitivity. The hypothalamus–pituitary–adrenal axis and the sympathetic corticosterone secretion by two different pathways: the sympathetic nervous system, whereas it controls the clock outputs, namely melatonin and corticosterone rhythms. Recently, it was reported that melatonin injection at the light/dark transition specifically increased SCN Per3 gene expression on the following day. Whether this effect of melatonin on the clock mechanism is present in all neurons, or in a specialized subset of neurons, and is responsible for the long lasting and specific effect on pineal activity remains to be determined.

In conclusion, the present study shows for the first time that a clock input, melatonin application at the light/dark transition, increases the amplitude of the endogenous rhythm of pineal melatonin without affecting the pattern of adrenal corticosterone release. As melatonin and corticosterone secretion are both under SCN control, it is suggested that exogenous melatonin
acts on a subpopulation of neurons controlling pineal melatonin secretion only. A further analysis of the mechanisms involved in the differential effect of melatonin will provide new insight into how the biological clock controls various physiological rhythms.

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