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Effects of melatonin on spontaneous electrical activity of neurons in rat suprachiasmatic nuclei: an in vitro iontophoretic study

Rapid Communication

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Summary. Circadian rhythms, endogenously generated in suprachiasmatic nuclei (SCN), seem to be under the direct influence of melatonin. Therefore, the effect of iontophoretically applied melatonin on electrical activity of SCN neurons was investigated in vitro. Usually, melatonin had an inhibitory effect. In the 3-h periods before (2.00–5.00 p.m.) or after (5.00–8.00 p.m.) the light-dark transition the percentage of SCN neurons sensitive to melatonin was very high (80% and 100%, respectively). However, efficacy of melatonin was low in the periods preceeding (20%) and following (33%) this 6-h time interval.

Keywords: SCN neurons, circadian rhythm, iontophoresis, melatonin receptors.

Introduction

The suprachiasmatic nuclei (SCN) are though to be the primary pacemaker for generation of endogenous circadian rhythms (Rusak and Zucker, 1979). In mammals, SCN lesions abolish circadian rhythmicity of various functions (Stephan and Zucker, 1974). Furthermore, SCN is the only part of mammalian brain capable of sustaining a circadian rhythm in electrical activity which persists in vivo when the SCN are neurally isolated from the rest of the brain (Inouye and Kawamura, 1982) and in in vitro in hypothalamic slice preparations (Green and Gillette, 1982; Groos and Hendricks, 1982; Shibata et al., 1982).

Circadian rhythms are entrained by the environmental light-dark regimen transduced via the retino-hypothalamic tract (Moore and Klein, 1974). Recently, it has been shown that free-running circadian rhythms in rat locomotor activity can be entrained also by the pineal hormone melatonin, but only, when it is applied at the beginning of subjective night (Redman et al., 1983). Since the effects of melatonin depend on the integrity of the SCN (Cassone et al., 1985),

it was proposed that melatonin entrains circadian rhythm by acting directly in the SCN. This assumption was further supported by autoradiographic detection of melatonin receptors in the SCN (Vanecek et al., 1987).

To further investigate site and mechanism of melatonin action, we applied this hormone iontophoretically while recording spontaneous electrical activity of single SCN neurons in vitro. Investigations were performed 6 h before to 6 h after the light-dark transition (5.00 p.m.), since within this period melatonin induced entrainment effects on running activity in rats (Redman et al., 1983).

Materials and methods

Adult male Sprague-Dawley rats (150–250 g b.wt.) were kept for at least 3 weeks under a light : dark regimen of 12 : 12 (lights on at 5.00 a.m.), with water and food ad libitum.

Animals were decapitated between 9.00 a.m. and 6.00 p.m. Brains were rapidly removed and sliced using a vibro-slice. Coronal hypothalamic sections (300–400 μm), containing SCN were placed in a Krebs-Ringer solution and preincubated for at least 0.5 h (37 °C; 80% O_2, 5% CO_2) before the tissue was transferred to a recording chamber. The activity of spontaneously active SCN neurons was recorded extracellularly through one barrel of conventional 3-barrelled glass microelectrodes (electrode resistance 12–20 MΩ), recording time from one and the same slice preparation not exceeding 8 h. The second barrel was used for balancing iontophoretic currents. Both, the recording and the balancing barrels contained 3 M NaCl. The third barrel was filled with a 10^{-3} M solution of melatonin. Ejecting currents ranged from 10–100 nA, ejecting time varied between 1–3 min. The amount of melatonin released by iontophoresis was in the range of 0.1–0.01 pmol as assessed by radioimmunoassay, quantity depending on electrode resistance, ejecting current and ejecting time (data not shown). This is about 1/10,000–1/100,000 the amount produced by rat pineal during one night (Illnerova et al., 1978). Between periods of melatonin ejection a cationic retaining current was applied (10 nA). Prior to iontophoretic application of melatonin, the discharge frequency of the neurons was observed for 3–5 min to assure frequency stability. Data processing of recorded action potentials has been described in detail elsewhere (Stehle et al., 1989). All cells recorded between 11 a.m. and 11 p.m. were further analysed. Melatonin-induced effect on spontaneous electrical activity of SCN neurons was considered to be substance-specific, provided it was reversible, reproducible and not mimicked by current ejection through the balancing barrel.

Results

Mean discharge rate of a total of 148 spontaneously active SCN neurons was analysed between 11.00 a.m. and 11.00 p.m. Discharge frequencies ranged from 0.1 up to 18 Hz. Firing pattern of the cells was regular (61%) or irregular. To enhance the evaluation, the frequencies of recorded cells within 3-h periods were pooled, starting at circadian time (CT) 6 (11.00 a.m.). Mean discharge rate decreased gradually over the 4 time intervals (Fig. 1A), with a significant difference of the 2 night-time values (CT 12–15; $P < 0.05$; CT 15–18: $P < 0.01$) as compared to the first day-time value (CT 6–9). This decrease in mean firing frequency of SCN neurons was not due to the extended period of in vitro incubation, as decrease was observed even in SCN preparations of freshly killed animals and regardless of the actual time of incubation or beginning of recording session.

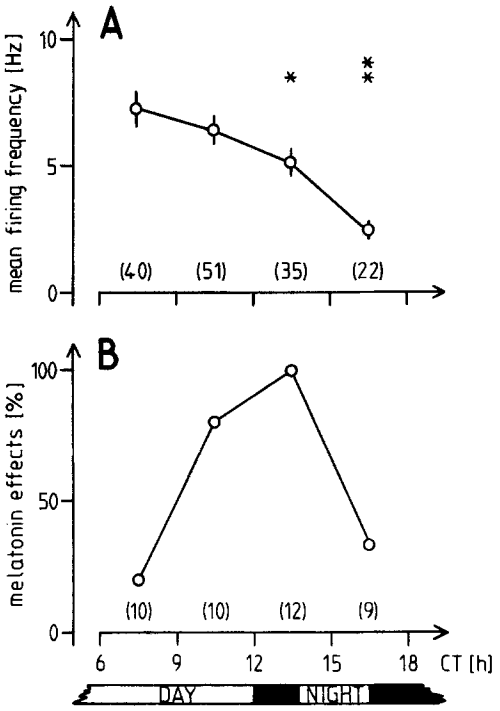

Fig. 1. A Frequency distribution of spontaneously active SCN cells with regard to time of day between CT 6 and CT 18, units subdivided into 3-h intervals. Asterisks indicate statistical significant difference to value obtained between CT 6 and CT 9 (** $P<0.01$; * $P<0.05$). Vertical bars indicate standard error of the means. **B** Percentage of SCN cells affected by melatonin with regard to time as indicated in Fig. 1 A. Number of cells are denoted in brackets

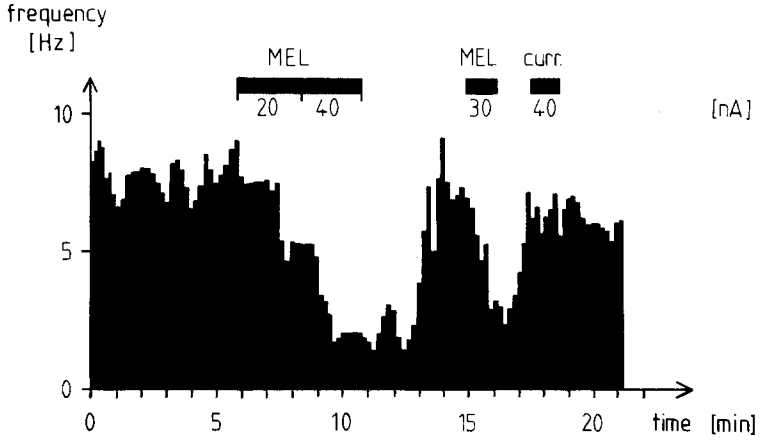

Fig. 2. Effect of iontophoretically applied melatonin (*MEL*) and of current (*curr.*) application on spontaneous electrical activity of an SCN cell

Iontophoresis of melatonin was conducted in 41 SCN neurons (28% of all SCN neurons recorded), 25 cells (61%) responsed to drug application, all but one cell exhibited a decrease in firing rate (Fig. 2). Between CT 6–9 unresponsive cells clearly outnumbered the cells that responded to melatonin (Fig. 1B). Sensitivity of SCN neurons' electrical properties to melatonin increased during the second period (CT 9–12), reached its peak in the first night-time period (CT 12–15) and decreased dramatically within CT 15–18 (Fig. 1B).

A typical example of the effect of melatonin on spontaneous electrical activity of an SCN neuron is shown in Fig. 2, where iontophoretic drug application reversibly inhibited the cells' firing rate in a dose-related manner. The effect outlasted melatonin application-time, the firing frequency returning within 3 min to the control level.

Discussion

The presented iontophoretic data show that melatonin exerts clear effects on SCN cells. Firing frequency of neurons affected was in all but one cell inhibited by application of the pineal hormone, with the efficacy of melatonin peaking around the day-night transition (CT 12). This period of highest sensitivity of SCN cells' electrical activity to melatonin correlates with the period when melatonin entrains free running rhythms in rat (Redman et al., 1983). This time interval coincides also with the inhibitory effects of melatonin on 2-deoxy-[1-^{14}C]glucose (2-DG) uptake in SCN (Cassone et al., 1988). Melatonin showed maximal effects on 2-DG uptake at the end of day (CT 10–11) and it had no effect 3 h after light off (CT 15). However, inhibitory effects of melatonin on 2-DG uptake occurred already as early as CT 6, a time point, where we observed only little efficacy of the hormone on electrical activity of SCN cells. These discrepancies might be due to differences between in vivo and in vitro preparations.

While this paper was in preparation, the inhibitory effect of melatonin on electrical activity of SCN neurons was shown by Mason and Brook (1988). We extend these findings and report a rhythm in sensitivity of SCN cells to application of melatonin, with its maximum at the beginning of rat's activity phase. The present and other data suggest that melatonin may have a pivotal role in the SCN which might mediate physiological effects of the pineal hormone including integration of circadian organisation in mammalian reproduction (Tamarkin et al., 1985).

Acknowledgements

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