
SHORT COMMUNICATION

Daily Rhythm in Rat Pineal Catecholamines

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Summary

A daily rhythm in the oscillations of pineal dopamine, norepinephrine and epinephrine content was found in male Wistar:Han rats. The acrophases of the oscillations were localized in the first half of the dark period and generally higher values were found in the dark part of the day.

Key words

Rat pineal • Catecholamines • Daily oscillations

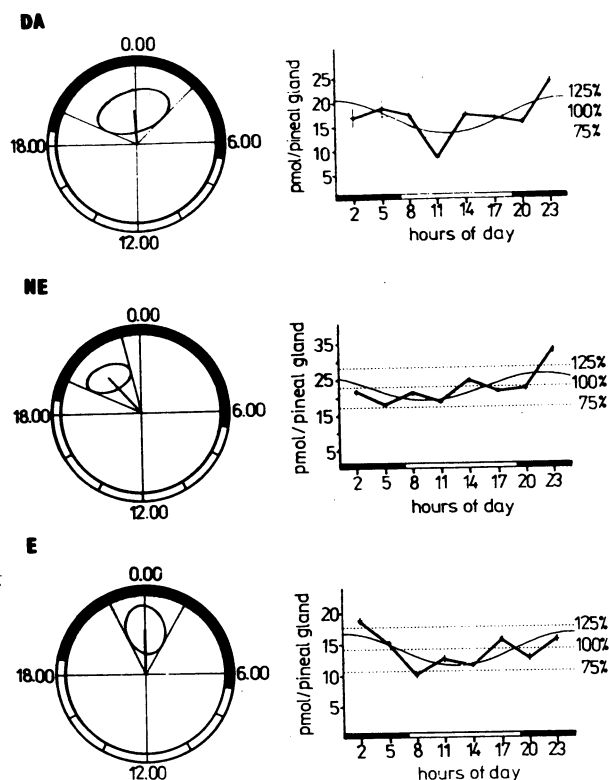
It is generally accepted fact the levels of pineal melatonin and of the activity of key enzymes of its synthesis, serotonin-N-acetyltransferase and hydroxyindole-O-methyltransferase are high during darkness, and the levels of pineal serotonin and its 5-hydroxyindole derivates are enhanced in the light period. However, other pineal substituents in rats are also distributed divergently through the light-dark cycle. The content of histamine (Sprague-Dawley rats, Garbar *et al.* 1974), the concentration of somatostatin (male rats, Peinado *et al.* 1990), the activities of thyroxine-5'-deiodinase (Sprague-Dawley rats, Murakami *et al.* 1989) and of aspartate aminotransferase (male Sprague-Dawley rats, Ramirez *et al.* 1992) are higher during darkness, and the circadian rhythm of lipid peroxides also peaks in this part of the day (Wistar male rats, Solár and Ahlers 1997). The concentration of cytosolic pinealocyte proteins (male Wistar rats, Nir *et al.* 1973a) and of free amino acids (the same animals, Nir *et al.* 1973b), especially that of taurine

(male Charles River rats, Piechowiak and Schnizer 1976) are higher during the light period.

Wurtman and Axelrod (1966) described a 24-hour rhythm in the content of rat pineal norepinephrine. As will be discussed later, there have appeared some other reports on pineal catecholamine rhythms. We were interested in the follow-up of daily oscillations in the content of dopamine, norepinephrine and epinephrine in the pineal gland of male Wistar:Han rats.

Male SPF bred Wistar:Han rats (Velaz Prague) weighing 200-250 g were adapted to a light:dark (LD) - 12:12 h regimen (light from 07 to 19 h), intensity of cold fluorescent light was 150 lux in the cage for three weeks, with free access to food and water. Animals were analyzed at 3 h intervals in the course of 24 h with beginning at 08:00 h; each of eight groups consisted of 7-8 rats. The pineal glands were removed, deeply frozen and later analyzed for dopamine (DA), norepinephrine (NE) and epinephrine (E) concentration according to

Johnson *et al.* (1980). Peritz's multiple F-test (Harper 1984) and population mean cosinor analysis (Halberg *et al.* 1967) were used for evaluation of the results.



The weight of the pineal gland did not change markedly during the period of observation. The content of pineal catecholamines oscillated in the course of 24 hours with a distinct rhythm and with acrophases in the first half of darkness (Fig. 1); other characteristics of the cosinor test are shown in Table 1. Generally, the average values from the dark part of the day were higher in comparison with "light" values, in the case of DA and E significantly (in pmol per pineal – DA: 19 ± 0.91 vs 14.7 ± 0.91 , $P < 0.01$; E: 15.44 ± 0.58 vs 12.33 ± 0.59 , $P < 0.01$; NE: 23.52 ± 1.29 vs 21.27 ± 0.65 , non-significant).

Fig. 1. Cosinor diagram (circular plots, left part) and circadian oscillations of pineal catecholamines: dopamine (DA), norepinephrine (NE) and epinephrine (E) - right part, values are given as means \pm S.E.M., content in pmol/pineal gland, stratified in % of overall means. Empty parts of basal band – light, full parts – darkness. The vector originating from the center of coordinates of the cosinor diagram represents the amplitude of oscillations. The orientation of the vector indicates the acrophase on the circular scale (in hours). The ellipse drawn with the center at the end of the vector represents the confidence area ($P < 0.05$) for the acrophase and amplitude. The tangents to the ellipse delimit the confidence interval for the acrophase ($P < 0.05$). The rhythm is present, if the ellipse does not overlap the origin of coordinates.

Table 1. Characteristics of the cosinor test

	Period of the rhythm (hours)	Mesor \pm S.E.M. (pmol/pineal gland)	Amplitude \pm CI (95 %) (pmol/pineal gland)	Acrophase \pm CI (95 %) (hours)
Dopamine	24	16.56 ± 0.53	3.44 (1.22; 5.52)	23.45 (19.37; 3.08)
Norepinephrine	24	22.38 ± 0.30	3.62 (2.52; 4.64)	21.13 (19.38; 23.02)
Epinephrine	24	13.89 ± 0.21	2.57 (1.18; 3.96)	24.00 (22.11; 02.00)

CI - confidence intervals; its limits are given in brackets.

Numerous data have documented the role of NE and DA in the signal transduction to pinealocytes in rats. The NE efflux was higher, but the turnover was lower during darkness in young Osborne-Mendel rats, the NE content was higher in the light and these differences persisted in blinded rats (Brownstein and Axelrod 1974). Endogenous tyrosine hydroxylase activity as a marker of pineal catecholamine synthesis was higher, as well as NE and DA levels, during the darkness in young male Sprague-Dawley rats kept under LD 14:10 h regimen (Craft *et al.* 1984). Pineal DA was higher during the dark period in Sprague-Dawley rats, but NE and E levels remained unchanged during nycthemeral arrangement of the experiment conducted by Hermes *et al.* (1994). The presence of E in the pineal gland is a matter for discussion. It may be derived principally from the adrenal medulla, but Saavedra *et al.* (1982) claimed that the rat pineal may be capable of E synthesis. The oscillations in pineal noradrenergic activity could be better explained by the circadian rhythm in terms of beta-adrenoceptor densities (Romero *et al.* 1975).

Experiments using long-day breeders and hibernators (hamsters) led to a new insight into the role of the catecholamines in the pineal gland. Indoleamines and catecholamines oscillated in the pineals of Syrian hamsters kept under long (LD 14:10 h) and short (LD 10:14 h) photoperiods with the exception of the NE content. A distinct increase in the DA content during darkness was observed, while it was lower under a short

photoperiod than under the long one (Miguez *et al.* 1995). No appreciable oscillations of pineal NE were noted in Djungarian hamsters kept under the long photoperiod, but a nocturnal DA increase was found irrespectively of the duration of the photoperiod (Miguez *et al.* 1996). DA does not seem to be only the precursor in NE synthesis, but also the inductor/stimulator of pineal melatonin synthesis, more so in hamsters than in rats (Moujir *et al.* 1997).

Some peculiarities of pineal catecholamines have to be mentioned when comparing the results of the above cited papers. Rat strains with different patterns of reactivity were used: Osborne-Mendel, Sprague-Dawley and Wistar:Han; the light-dark regimen oscillated between 12:12 h in rats to 14:10 h in some experiments with hamsters. The data resulted from sampling at various times of the day, e.g. only one sample from light and dark (Hermes *et al.* 1984), two samples from both parts (Brownstein and Axelrod 1974) or, in our case regular sampling at 3-h intervals during 24 h, more suitable for the statistical evaluation of the oscillations. Therefore, a comparison of individual or average values, definition of the highest and lowest points obtained at different times of the day, in different strains of animals and under different experimental conditions is difficult.

Our report has demonstrated rhythmic daily oscillations of pineal catecholamines in male Wistar:Han rats, with higher average values during the dark part of the day.

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