CIRCADIAN RHYTHM OF PLASMA TESTOSTERONE IN THE MALE DJUNGARIAN HAMSTER (PHODOPUS SUNGORUS)

By

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ABSTRACT

A marked circadian rhythm of plasma testosterone was found in male Djungarian hamsters. Maximal values in the evening just prior to activity onset were about 12 times higher than minimum values in the early morning. The peak of serum testosterone concentration coincides with onset of oestrus in females.

During the last decade circadian rhythms have been described for many hormones in man as well as in other mammals (Aschoff et al. 1974; Krieger & Aschoff, in press). In plasma testosterone circadian rhythms have been reported in man (Nieschlag 1974; Rubin et al. 1975), in monkeys (Michael et al. 1974; Goodman et al. 1974; Mukku et al. 1976) and in several ungulates (Wetteman & Desjardins 1973; Ellendorff et al. 1975; Kirkpatrick et al. 1976). In rodents a circadian rhythm has been reported for the laboratory rat, but here the findings are contradictory and no consistent picture ensues (Kinson & Liu 1973a,b; Howland 1975; Wilson et al. 1976). It was therefore desirable to examine the daily fluctuation in plasma testosterone in another rodent species.

Supported by Deutsche Forschungsgemeinschaft, Schwerpunktprogramm Biologie der Zeitmessung.
Under natural conditions many mammals show a marked annual variation in gonadal size and activity. In such species comparison of hormone levels at different seasons could be misleading if a circadian rhythm existed and was not taken into consideration. The Djungarian hamster *Phodopus sungorus* shows a distinct annual cycle of gonadal size and activity if kept under natural light conditions (Fig. 1), and reacts strongly to changes in photoperiod (*Hoffmann* 1973, 1974; *Hoffmann* & *Küderling* 1975). Details of the circadian fluctuation of plasma testosterone in summer animals of this species are presented in this paper.

**MATERIAL AND METHODS**

The animals used in the study reported here belong to the subspecies *Phodopus s. sungorus* Pallas which has been bred in our laboratory since 1968. The stock originates from four animals caught near Omsk in Siberia (*Figala et al.* 1973). Determination of plasma testosterone was performed in spring at a time when testes were large and in full spermatogenesis (see Fig. 1). The males were kept singly in plastic cages (29 x 19 x 14 cm) from weaning on and were housed in our breeding room (temperature 20° ± 3°C) which is exposed only to natural light conditions throughout the year (for further details of animal care see: *Figala et al.* 1973). The animals weighed about 45 g.

Blood from a total of 240 males was collected on three days (from April 29 to May 2, 1976). The animals were between 280 and 357 days old. They were removed singly from the animal house, injected with about 500 USP-E. natrium heparinate (Liquemin) to increase yield of blood, and anaesthetized with halothane. The thoracic cavity was opened and blood was obtained by heart puncture. Not more than 5 min elapsed between removal from animal quarters and blood collection. In all cases it was ascertained that both testes were fully active by inspection of smears from the

![Graph](https://via.placeholder.com/150)

*Fig. 1.*

Annual cycle of testis weight in *Phodopus* living in natural photoperiods. Each point represents the mean for 15 males, standard error is given by vertical bars. The histological examination showed corresponding seasonal changes. Values for January to March have been repeated to facilitate inspection.
caudae epididymidis which were packed with motile spermatozoa. The blood was allowed to cool at 4°C for several hours and centrifuged for 12 min at 2800 r.p.m. About 0.5 ml plasma was thus gained per animal, frozen immediately, and stored at -20°C until assayed.

The blood of 10 hamsters was collected within 80 min after each of the following hours: 9 h, 12 h, 15 h, 18 h, 21 h, 24 h, 3 h, and 6 h. This collection procedure was performed on each of three consecutive days, thus yielding blood samples from 30 animals per time of day. Samples of 1 ml plasma, pooled from 1 to 3 (normally from 2) males were assayed. The remaining plasma was used for determination of the percentage binding of testosterone to plasma proteins.

A pilot experiment using only 10 animals for each time of day had been performed in July 1975 (24th to 31st) with males between 290 and 433 days old. Pooled plasma samples from the 10 hamsters sacrificed at each time were assayed for testosterone, thus yielding one value per time of day. All other procedures were the same as those reported above.

Plasma testosterone was measured by a specific radioimmunoassay after isolation by thin layer chromatography (Nieschlag & Loriaux 1972). The percentage binding of testosterone was determined by equilibrium dialysis at 37°C according to Forest et al. (1968).

Fig. 2.
Circadian cycle of plasma testosterone concentration. Means and standard errors are given. Small dots give values of single determinations. Times of light and darkness are indicated by white or black bar underneath; skewed margins of black bar indicate time of civil twilight. SS = sunset, SR = sunrise.
RESULTS

Plasma testosterone in the hamsters showed a marked daily cycle (Fig. 2) with high values during daytime, a pronounced peak in the evening and low values during the night. The value at 18 h is significantly higher than the values obtained at any of the other times ($P < 0.01$ to $< 0.001$, t-test), the values at 9 h, 12 h, and 15 h differ significantly from those obtained at 24 h, 3 h, and 6 h ($P < 0.05$ to $< 0.001$). The average value from all 8 times was 1.18 ng/ml plasma, the peak in the evening was more than 3 times the average value, or more than 12 times higher than the minimum value obtained at 6 h in the morning. Daytime values at 9 h, 12 h, and 15 h were in general 2 to 3 times higher than those during the night and the early morning (24 h, 3 h, and 6 h).

Since the sampling was performed on three consecutive days, it is unlikely that the peak obtained at about 18 h is due to some unspecified disturbance. The result of the pilot experiment performed in summer one year earlier further emphasizes that the temporal pattern found is representative (Fig. 3). Here an even higher peak value was obtained in the evening, and the lowest value was also found in the early morning. Thus the data given in Fig. 2 seem representative for the species during the summer months when the testes are large and active.

The values for bound testosterone varied between 96.3 % and 97.7 %, and no marked differences at different times of day were discernible. This means that the higher values during daytime and especially the peak value in the evening also represent higher amounts of free testosterone.

![Fig. 3.](image)

Daily variation of plasma testosterone in pilot experiment in July 1975. Each dot represents value for pooled plasma from 10 animals. SS = sunset, SR = sunrise.
Fig. 4.
Locomotor activity of a male Djungarian hamster kept in the breeding room under natural light conditions, April 23 to May 10. The animal was housed in a cage with a running wheel, each rotation of the wheel activated an event recorder, black bars thus represent times of intensive activity. Daily recordings have been pasted underneath each other. Sunset (SS) and sunrise (SR) for May 1 is indicated.

DISCUSSION

The results demonstrate a daily rhythm in plasma testosterone with higher values during daytime than at night and in the early morning, and with a pronounced peak in the evening before sunset. This is just before the time when the animals start their nocturnal activity, as recordings of locomotor activity demonstrated (Fig. 4). In female golden hamsters (Mesocricetus auratus) it was found that behavioural oestrus (onset of heat) begins at about 18 h if the animals are kept in an artificial light-dark cycle with light from 4 to 20 h (Alleva et al. 1971), a schedule roughly corresponding to that of the natural light cycle in our experiments (see Fig. 2). In the golden hamster it was also shown that under constant conditions oestrus starts about 1 to 2 h before normal onset of activity (Morin et al. 1977). In Phodopus no systematic study of onset of oestrus was carried out, but observation of copulations in breeding pairs suggest that oestrus in this species also begins before sunset, and onset of main nocturnal activity. One might speculate that the marked rise in testosterone in males at this time has functional significance and enhances sexual behaviour though such rapid action of testosterone on behaviour has not yet been described. In male golden hamsters in breeding condition a maximum of plasma LH concentration was reported at about that time (Turek et al. 1976). – The rather large scatter of values we found during daytime and especially in the evening suggests episodic secretion of testosterone at these times.

The hamsters used in our experiment were housed in the breeding room where breeding pairs and some single females were also kept. Although the cage with these animals were on different shelves, some meters distant from the experimental animals, it cannot be excluded that stimuli released from the females at onset of heat, e. g. olfactory signals (pheromones), are responsible
for the sharp rise of plasma testosterone in the males at that time. If this were the case, such stimuli should be released by the females only at the time of onset of oestrus, or only be responded to by the males at that time, since the peak in testosterone is limited to a rather short time while oestrus lasts much longer, up to 18 h in the golden hamster (Alleva et al. 1971).

In other mammalian species in which a circadian rhythm of plasma testosterone was described there is some variation in the time of day at which maximal and minimal values were found. In man, pig, and horse, higher values in the morning than in the evening were reported (Nieschlag 1974; Ellendorff et al. 1975; Kirkpatrick et al. 1976); in monkeys and in the ram higher values were found during the day than at night (Michael et al. 1974; Goodman et al. 1974; Mukku et al. 1976; Wetteeman & Desjardins 1973). All these species can be considered to be active mainly during the day while Phodopus is active at night (see Fig. 4). The higher values during resting time in Phodopus would thus correspond to the higher nocturnal values found in the species mentioned above. In the only other rodent studied so far, the dark-active laboratory rat, the times for which maximal and minimal plasma testosterone values were reported vary in different studies (Kinson & Liu 1973a,b; Howland 1975; Wilson et al. 1976). The reason for this inconsistent picture is unclear. Some of the findings suggest a change of pattern with age; however, other factors like housing of animals, season, sexual experience, strain of rats or way of blood collection might also be involved.

In none of the cases in which a circadian rhythm of plasma testosterone was reported, a sharp and high maximum was found comparable to the evening peak in Phodopus; however in bulls episodic release with similar steep rises has been reported (Katongole et al. 1971). But it must be kept in mind, that in several of the papers quoted only 4 determinations per day or even less were performed (Michael et al. 1974; Goodman et al. 1974; Mukku et al. 1976; Kinson & Liu 1973b; Howland 1975). If, in Phodopus, blood samples had been collected only at four times of day, e.g. at 3 h, 9 h, 15 h, and 21 h, the peak in the evening would have remained unnoticed, and only higher values during the day than during the night would have been found.

Except for the high values of the evening peak, the plasma testosterone concentrations in Phodopus lie in the lowest range of values reported so far for mammalian species (Gustafson & Shemesh 1976). In the golden hamster values of about 5 to 9 ng/ml were reported in males kept in long photoperiods (Berndtson & Desjardins 1974), however, the hour of blood collection was not specified in these experiments.

Note added in proof
Additional experiments with male hamsters kept in artificial long days (light from 4 to 20 h) and without access to female odour gave a similar peak of plasma testosterone
before onset of dark (15 h: 2.83 ± 1.42 ng/ml; 18 h: 5.71 ± 1.79 ng/ml; 21 h: 2.84 ± 1.86 ng/ml). Stimulation by female odour is thus not responsible for the sharp rise of plasma testosterone at that time.

ACKNOWLEDGMENTS

We are indebted to Miss A. Bock, Miss D. Janson-Smith, Miss A. Kolbe, and Dr. M. Brackmann for their cooperation in obtaining the blood samples at rather inconvenient hours.

REFERENCES


Received on December 24th, 1976.