Seasonal changes in testicular contents of testosterone and androstenedione and in the metabolic clearance rate of testosterone in the sand rat (*Psammomys obesus*)

Farida Khammar and R. Brudieux†

Laboratoire d'Endocrinologie et Ecophysiologie animale, Centre National de Recherches sur les Zones arides (O.N.R.S.), B.P. 119, rue Danton, Alger, Algérie

Summary. Adult male sand rats (*Psammomys obesus*) were caught in the Béni-Abbès area. The highest testicular contents of androgens (ng/testis) were observed in autumn and in winter (testosterone: 7·6 ± 1·1; androstenedione: 0·76 ± 0·11) and the lowest in early summer (June) (testosterone: 1·5 ± 0·3; androstenedione: 0·20 ± 0·05). Values had increased by late July. Annual variations of the testosterone metabolic clearance rate (litres/24 h/100 g body wt) were similar to those of testicular androgens; values were high in winter (6·7 ± 0·7) and lowest in June (3·2 ± 0·3). The onset of testicular endocrine activity in sand rats was concomitant both with the highest temperatures and the start of reduction in photoperiod; its regression occurred when temperature and photoperiod were increasing.

Introduction

The annual reproductive cycle of many species of wild mammals, mainly in Europe and North America, has been extensively studied. In contrast, work on desert mammals is scarce and refers mostly to ecological observations (Charnot, 1964; Reichman & Van de Graaf, 1973; Happold, 1975; Misonne, 1975; Naumov & Lobachev, 1975; Prakash, 1975; Smith & Jorgensen, 1975). We have found nothing in the literature concerning the production of androgens by the testis of desert rodents.

In a previous report (Amirat, Khammar & Brudieux, 1977), we have shown that the sand rat (*Psammomys obesus*), a gerbilline inhabiting underground burrows on briny alluvial platforms of the Sahara desert, exhibited, in the field, a seasonal reproductive activity: pregnancies occurred from October to May and testicular and seminal vesicle weights were fully developed in autumn and winter, and decreased in spring.

In the present study we have investigated the seasonal changes of secretory function of the testis of the sand rat by measuring testicular testosterone and androstenedione contents and the metabolic clearance rate of testosterone.

Materials and Methods

**Animals.** Sand rats (*Psammomys obesus*) were obtained from the Béni-Abbès area (Wilaya of Béchar, Algeria: 30°7′N, 2°10′W) in the Sahara desert. Adult males were live-trapped in the field

* Reprint requests to Dr F. Khammar.
† Present address: Laboratoire d'Endocrinologie comparée, U.E.R. de Biologie, Université de Bordeaux I, avenue des Facultés, 33405 Talence Cédex. France.
approximately every 45 days; the adult condition was checked according to the criteria previously described (Amirat et al., 1980).

The 79 sand rats, caught from December 1977 to November 1978 (i.e. 28 December 1977-2 January 1978; 24 February-2 March 1978; 10-14 April 1978; 2-5 June 1978; 17-22 July 1978; 17-23 September 1978; 19-25 October 1978), were, immediately after trapping, kept for 12-24 h in individual cages in the laboratory and fed exclusively fresh *Suada mollis, ad libitum*; this is a succulent salty plant of the Chenopodiaceae family which is the foodstuff of the sand rat in this biotope and provides sufficient water. The sand rats were killed by decapitation, always in the late morning. Blood from the neck was collected on calcium heparinate and immediately centrifuged. Seminal vesicles and testes were quickly removed and weighed. The testes and plasma were immediately frozen and carried in liquid nitrogen to Alger, where they were stored at −25°C until assay for testosterone and androstenedione.

Another 92 sand rats were trapped from June 1979 to September 1980 (i.e. 6-26 June 1979; 9-25 October 1979; 1-7 December 1979 + 4-12 January 1980; 1-8 March 1980; 1-6 May 1980; 11-16 June 1980; 14-16 July 1980; 28-30 September 1980); they were kept in the laboratory, in the same conditions as above, until infusion for determination of the metabolic clearance rate of testosterone, carried out always between 08:00 and 13:00 h.

**Hormone assays.** The testosterone and androstenedione contents were measured, by radioimmunooassays, in the same samples, according to a technique similar to that previously described by Darbeïda & Brudieux (1980) for plasma testosterone and 5α-dihydrotestosterone concentrations, after extraction by ethyl acetate–cyclohexane and purification on celite columns. Slight differences in the techniques used are indicated below.

The testosterone antibody was prepared against testosterone carboximethylxolime–BSA (Darbeïda & Brudieux, 1980) and used at a dilution of 1:40 000. The androstenedione assays were performed with an antiserum from Institut Pasteur (Paris, France).

The left testis of each animal was thawed and minced in 8 ml twice-distilled water. An appropriate volume of homogenized testis, to which was added 1000 c.p.m. [1,2,6,7-3H]testosterone (sp. act. 85 Ci/mmol) and 1000 c.p.m. [1,2,6,7-3H]androstenedione (sp. act. 114 Ci/mmol) (New England Nuclear Corporation, Boston, U.S.A.) for recovery, was extracted once with 3 volumes of ethyl acetate–cyclohexane (50:50, v/v). Celite columns were 5 ml Kimble disposable pipettes (Owens, U.S.A.), sealed with a glass bead, containing 0.8 g of a mixture of 0.5 g celite 535 (Touzart et Matignon, Paris, France) and 0.25 ml propylene glycol, packed in a vacuum and then saturated with 30 ml iso-octane. Elution from celite columns was carried out stepwise using 4 ml iso-octane, 4 ml iso-octane–benzene (82:8, v/v), 4 ml iso-octane–benzene (70:30, v/v) and 4 ml iso-octane–benzene (40:60, v/v). The first and the third fractions were discarded; the second fraction contained androstenedione and the last fraction testosterone. The dried residue of each hormone fraction was dissolved in 2-0 ml (testosterone) or 0-4 ml (androstenedione) phosphate buffer.

Radioimmunoassays were carried out on duplicate 0-1 ml samples, using 4000 c.p.m. [3H]testosterone or [3H]androstenedione, 0-1 ml antibodies and 1 ml cold charcoal-coated dextran solution. Incubation was performed at +4°C during 3 h for testosterone and during 30 min at room temperature followed by 15 min at 0°C for androstenedione. The results, corrected for recovery, dilutions and water blank, were expressed as ng/testis for both hormones.

Because of the purification step by celite columns and the specificity of the antisera the method used was specific; it was also sensitive (after logit-log transformation, standard curves were linear between 5 and 500 pg for both hormones; blank values were 7-3 pg/tube for testosterone and 10-2 pg/tube for androstenedione; mean recoveries were 65±1 ± 1-3% for testosterone and 70-0 ± 2-0% for androstenedione), precise (within-assay variances: 9-4% for testosterone and 12-2% for androstenedione; between-assay variances: 9-1% for testosterone and 10-2% for androstenedione) and accurate (the correlation coefficient between estimated and expected values was 0-999 for both hormones).
Metabolic clearance rate of testosterone. Each sand rat was anaesthetized with an i.p. injection of 4 mg pentobarbitone sodium/100 g body wt (Nembutal; Abbott, Saint-Rémy-sur-Avre, France) and heparinized with an i.v. administration of 50 i.u. (0·2 ml) calcium heparinate/100 g body wt. About 1 µCi/h/100 g body weight of [1,2,6,7-3H]testosterone dissolved in 0-9% (w/v) NaCl was continuously infused through the right jugular vein at a constant rate of 0·195 ml/h; steady state plasma concentrations of [3H]testosterone were obtained within 40 min after the start of the infusion. Then 5 × 0·25 ml blood samples were withdrawn from the carotid artery every 10 min between 50 and 90 min after the start of infusion. Plasma samples were stored at -25°C until analysis.

After addition of 400 c.p.m. [1,2-14C]testosterone to each tube, to determine the recovery, testosterone was extracted and purified according to the technique described above for measurement of testicular content of testosterone.

The radioactivity of the dried testosterone fractions was determined by liquid scintillation counting with dual isotope setting using a scintillation fluid containing 4-0 g 2,5-diphenyl-oxazole + 0·04 g p-bis-2 (5-phenyl-oxazolyl)benzene in 1000 ml toluene. The metabolic clearance rate of testosterone (l/h) is the ratio of infusion rate (d.p.m./h) to equilibrium plasma concentration (d.p.m./l) of [3H]testosterone.

Statistical analysis. Means and standard errors were calculated and the significance of the difference was determined by Student's t test.

Results

Testicular and seminal vesicle weights

Data reported in Table 1 show that the testicular and seminal vesicle weights exhibited similar marked seasonal variations in 1978 and in 1979–1980. The most elevated weights occurred in autumn and in winter; they declined from April, reaching a minimum in late spring and early summer (June–July).

The overall decreases (P < 0·001) were -67-7% in 1978 and -63-3% in 1980 for testicular weights and -86-3% in 1978 and -77-6% in 1980 for seminal vesicle weights.

<table>
<thead>
<tr>
<th>Table 1. Seasonal changes in body weight, testicular and seminal vesicles weights in adult male sand rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>1978</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1979–1980</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Text-fig. 1. Seasonal changes in testicular contents of testosterone and androstenedione in adult male sand rats. Values are means ± s.e.m. for the number of animals indicated.

Testicular contents of testosterone and androstenedione

The hormone changes in the testes are shown in Text-fig. 1.

Testosterone. Values ranged between 1·5 and 7·6 ng. They exhibited important and statistically significant seasonal changes: testicular testosterone levels were 6·2 ± 0·4 ng in January 1978; a modest decline occurred in April (−19·5%; \( P < 0·02 \)); the minimum values (1·5 ± 0·3 ng) were in early June; and then there was a marked increase in July (+173·3%; \( P < 0·001 \)) and a maximum in September (+406·6%; \( P < 0·001 \)).

Text-fig. 2. Seasonal changes in metabolic clearance rate (MCR) of testosterone in adult male sand rats. Values are means ± s.e.m. for the number of animals indicated.
Androstenedione. Mean testicular contents of androstenedione were an order of magnitude lower than those of testosterone; values ranged between 0·20 and 0·74 ng but the seasonal changes were similar to those of testosterone. The high values observed from January to March (0·67 ± 0·05 and 0·68 ± 0·10 ng) showed a slight reduction in April (−19·4%; P < 0·2); minimum values occurred also in June (−62·9%; P < 0·001); increasing in July (+60·0%; P < 0·05) and reaching maximum (+270·0%; P < 0·001) in September.

Metabolic clearance rate of testosterone

The MCR of testosterone (l/24 h) varied significantly throughout the year (Text-fig. 2). The seasonal changes were characterized by the highest values (about 10 l/24 h) in winter, and lowest values in early summer (2·5 ± 0·3 and 4·3 ± 0·4 l/24 h, in June 1979 and in June 1980 respectively). The decrease (−56·1%) between January and June 1980 was statistically significant (P < 0·001). MCR then rose slightly in July (+21·8%; P < 0·05), but markedly in September (+81·4%; P < 0·001). The MCR increase from June 1979 to December 1979 + January 1980 was +292·0% (P < 0·001).

When MCR was expressed as l/24 h/100 g body weight, it showed the same pattern; the January to June decrease (−52·2%) and the June to September increase (+100·0%) were statistically significant (P < 0·001).

Discussion

Testicular contents of testosterone in the sand rat (1·5—7·6 ng/testis, i.e. about 1·7—3·5 ng/100 mg testis wt) were similar to those in the gerbil (Gerbillus gerbillus), a desert rodent inhabiting the same area (Kassir, Khammar & Brudieux, 1980; unpublished data), but they were lower than those of laboratory rodents such as mice (50—70 ng/100 mg testis wt: Jean-Faucher, Berger, de Turckheim, Veyssière & Jean, 1978), rats (about 10 ng/100 mg testis wt: Knorr, Vanha-Pertulla & Lipsett, 1970) and guinea-pigs (17·9 ng/100 mg testis wt: Rigaudière, Pelardy, Robert & Delost, 1976) and those of rabbits (10 ng/100 mg testis wt: Berger et al., 1979).

The testicular content of androstenedione in the sand rat was low (0·20—0·74 ng/testis, i.e. about 0·20—0·34 ng/100 mg testis wt). The androstenedione to testosterone testicular ratio was around 0·1. This ratio is below 1 in adult rodents, but in sand rats it was lower than in other species (mouse: 0·66, Berger, Jean-Faucher, de Turckheim, Veyssière & Jean, 1975; guinea-pig: 0·30, Rigaudière et al., 1976). Nevertheless, testicular contents of testosterone and androstenedione exhibited marked significant annual variations in wild-caught sand rats, being highest in autumn and winter and lowest in early summer. These patterns of changes paralleled those of testicular and seminal vesicle weights from the same animals (Table 1), which agreed with previous observations in 1974 (Amirat et al., 1977).

Because only a small part of the testis consists of Leydig cells and there is probably a seasonal pattern of spermatogenesis, it was meaningless to express seasonal changes of hormone concentrations per unit testis weight. Our results do, however, strongly suggest that the secretion of testosterone undergoes marked seasonal variations in the sand rat.

Using the present radioimmunoassay method, we failed to measure precisely the plasma concentration of testosterone of the sand rat, because of the small blood volume collected after decapitation and the very low plasma level of testosterone which was only about 100 pg/ml even during the breeding season, compared with values of 5 ng/ml in the mouse (MacKinney & Desjardins, 1973; Jean-Faucher et al., 1978), 2—4 ng/ml in the rat (Resko, Feder & Goy, 1968; Corpechot, Beaulieu & Robel, 1981) and the rabbit (Berger et al., 1979), 3—5 ng/ml in the guinea-pig (Rigaudière et al., 1976) and 2—4 ng/ml in the Ouled Djellal ram in Algeria (Darbeida & Brudieux, 1980).
The low plasma concentration of testosterone of the sand rat is probably not the result of a high metabolic clearance rate: It was $3.2 \pm 0.3$ and $6.7 \pm 0.7$ 1/24 h/100 g body weight in June and December-January respectively compared to 17.51/24 h/100 g in the rat (Lee, Bird & Clark, 1975), 39.11/24 h/100 g in the rabbit (Mahoudeau, Corvol & Bricaire, 1973) and 5.31/24 h/100 g in the guinea-pig (Robert & Delost, 1978). Nevertheless, in the sand rat, the testosterone metabolic clearance rate exhibited marked seasonal variations: it was high in winter, decreased in spring, was low in June and then increased again. Therefore, the sand rat is like the fox (Maurel & Boissin, 1982), monkey (Wickings & Nieschlag, 1977), hedgehog (Saboureaux, 1979) and ram (Darbeida & Brudieux, 1980), in which seasonal changes in the metabolic clearance rate of testosterone paralleled those of the endocrine activity of the testis.

It will be necessary to investigate whether, as in adult foxes and badgers (Maurel & Boissin, 1982), seasonal changes in the metabolic clearance rate of testosterone in sand rats are related to annual variations in the linkage capacity of the specific steroid-binding protein which might be reduced during the period of sexual activity (autumn and winter), thus increasing the plasma concentrations of free testosterone and hormone catabolism. Environmental factors such as temperature, photoperiod and nutrition also need to be examined in detail. Succulent salty plants such as Suaeda mollis, are always available throughout the year in the sand rat biotope, but seasonal changes in composition may occur.

In sand rats the onset of testicular activity was concomitant both with the highest temperatures and the start of reduced daylength; its regression occurred when temperature and photoperiod were increasing (Text-fig. 3).

![Text-fig. 3. Annual variations in air temperature (○ maximum; ● minimum) and photoperiod (■) in the Béni-Abbès area (30°07'N; 2°10'W) between December 1977 and November 1978.](image)

**References**


Received 20 September 1983