Changes in the concentrations of testosterone and androstenedione in the plasma and testis of the guinea-pig from birth to death

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Summary. Testosterone and androstenedione concentrations in the plasma and testis of male guinea-pigs were estimated by gas chromatography at intervals (36 stages) from birth to death. Four main periods of androgenic activity were recognized. The neonatal period, from birth to Day 16, is characterized by a precocious but transient peak in plasma testosterone concentration at Days 2 and 3. The pubertal period from Days 16 to 90 can be subdivided into a prepubertal period starting on Day 16 and marked by a sudden linear increase in plasma and testicular testosterone concentration together with an increase in testicular and seminal vesicle weight, and a postpubertal period, from Day 50 (the time of hormonal puberty) to Day 90, characterized by high and stable androgen levels while testicular and genital tract development continues. Adulthood spans the period between Months 3–6 and Month 24; plasma and testicular concentrations of testosterone and androstenedione are stable but lower than those observed during puberty. The period of senescence occurs between Months 24 and 28 and is marked by a fall in testosterone secretion and involution of the seminiferous tubules and accessory sex glands.

Introduction

Recent studies on male mammals have shown that the hormonal secretions of the testis change during the life of an animal, particularly during specific periods of reproductive activity. For example, relatively high androgen levels have been recorded at birth in the plasma and testis of man (Forest, Cathiard & Bertrand, 1973a, b), monkey (Resko, 1970b), rat (Döhler & Wuttke, 1975) and lamb (Attal, 1970). During puberty the amount of androgens increases in different compartments in man (Frasier, Gafford & Horton, 1969; August, Grumbach & Kaplan, 1972; Knorr, Bidlingmaier, Butenandt, Fendel & Ehart-Wehle, 1974), rat (Knorr, Vanha-Perttula & Lipsett, 1970; Döhler & Wuttke, 1975; Gupta, Zarzycki & Rager, 1975b), mouse (McKinney & Desjardins, 1973) and lamb (Courrot, 1971). Androgen concentrations during adulthood and senescence have been particularly well studied in man (Vermeulen, Rubens & Verdonck, 1972; Nieschlag, Kley, Wiegmann, Solbach & Kruskemper, 1973; Pirke & Doerr, 1973; Stearns *et al.*, 1974): there is a notable decrease in old age from the stable levels in the adult. However, no investigation has so far been made on the secretion of androgens between birth and death of animals of the same species. Only such a study can provide precise information on the critical stages of transition between the main periods of androgenic activity. We have therefore studied the changes in plasma and testicular testosterone and androstenedione concentrations from birth to death in the guinea-pig, a species for which there is little information (Rivarola, Snipes & Migeon, 1968; Resko, 1970a).

Materials and Methods

Animals

The study was carried out on about 1000 male guinea-pigs of the Dunkin–Hartley strain which were born and bred in the laboratory. Newborn animals of both sex remained with the mother until

weaning on Day 20. Young males were then kept together until 3 months of age. Adults were regularly used for breeding from 3 to 24 months of age. The animals were killed by decapitation. Blood was collected from the neck, centrifuged and deep frozen at -35°C , as were the testes. Some gonads and seminal vesicles were used for histological study. Newborn animals were killed at 0, 6, 12, 18 and 24 hr after birth. The others were killed between 09.00 and 11.00 hours on Days 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 20 of age during the neonatal period; on Days 30, 40, 45, 50, 55, 60, 70, 80 and 90 of age during puberty; and at Months 6, 9, 18, 20, 22, 24, 28, 31 and 35 for adulthood and senescence. Death for Dunkin-Hartley male guinea-pigs was estimated by a statistical study to occur on average at 25 months of age.

Assays

Concentrations of testosterone and androstenedione were estimated by gas-liquid chromatography with electron capture detection (tritiated source) according to the micromethod of Attal (1970): unconjugated hormones were extracted with ether; testosterone and androstenedione were separated by thin-layer chromatography; androstenedione was reduced to testosterone with sodium borohydride; testosterone was converted to testosterone-17 β heptafluorate and then measured with an F and M Biochemical 402 chromatograph. The method gave recovery values of known amounts of [1,2- ^3H]testosterone and [1,2- ^3H]androstenedione (sp.act. 40–50 Ci/mmol: New England Nuclear Corporation) of 40% for testosterone and 20% for androstenedione; sensitivity was 1 ng for testosterone and 5 ng for androstenedione. Precision was given by a coefficient of variation $< 10\%$ for quantities higher than 5 ng for the two hormones. Androgens were measured in the testes of each animal and 10–20 estimations were made at each stage. Plasma androgens were estimated on pooled samples of plasma (2–8 animals in each pool) in the 0 hr to 20-day-old animals and on 10–20 individual samples in the others. Concentrations are expressed as ng/10 ml plasma and as ng/2 testes. Means are given with the standard error (S.E.M.); probability and significance were calculated by Student's *t* test.

Results

Changes in body, testicular and seminal vesicle weight

These are shown in Text-fig. 1; the values were more or less parallel throughout life. Testicular weight increased linearly ($y = 49.13x - 705.19$; $r = 0.99$) between Days 16 and 90 of life. Thereafter the weight increased slightly until Month 6, remained stable until Month 28, and then decreased by 22% ($0.02 < P < 0.05$). Between Day 18 and Month 6, the linear increase of seminal vesicle weight was marked ($y = 24.04x - 183.48$; $r = 0.96$). The weight continued to increase from Months 6 to 18 (34%, $0.02 < P < 0.05$), then steadily decreased by 43% at Month 35 ($P < 0.001$).

Histology

In most individuals, the first spermatozoa appeared in the seminiferous tubules between Days 55 and 60. Spermatogenesis was regular until Month 22. Thereafter disruptions could be seen in the seminiferous epithelium and about 25% of tubules were beginning to involute although epididymal sperm reserves appeared normal. From Month 31 involution affected 50% of tubules (diminution in germ cells, elimination of the multinucleated cells of degeneration, deformation of the tubule) and was followed by a depletion of the spermatozoa in the epididymis. Nevertheless, at this age spermatogenic activity remained apparently normal in 50% of animals. Histological study of the seminal vesicles showed that the epithelium was well stimulated until Month 24. At Month 28, slight involution appeared in 30% of the animals. By Month 31 65% of seminal vesicles displayed a poorly stimulated or atrophied epithelium and at Month 35 the seminal vesicles of all animals were atrophied.

Text-fig. 1. Changes in mean $\pm$ S.E.M. weight of the body (○), testes (●) and seminal vesicles (▽) of guinea-pigs from birth to death.

Text-fig. 2. Changes in mean $\pm$ S.E.M. concentrations of plasma testosterone (●) and androstenedione (○) in the guinea-pig from birth to death.

Text-fig. 3. Changes in mean $\pm$ S.E.M. concentrations of testicular testosterone ($\bullet$) and androstenedione ($\circ$) in the guinea-pig from birth to death.

Changes in plasma concentrations of testosterone and androstenedione

These are shown in Text-fig. 2. Testosterone concentration on Day 3 was significantly higher than that at 12 hr ($P < 0.001$) and on Day 4 ($0.02 < P < 0.05$). No similar increase of androstenedione was observed. From Day 4, the two androgens behaved differently: the plasma androstenedione level regularly increased until Day 60, while plasma testosterone levels remained stable between Days 4 and 16, then increased rapidly until Day 60. Maximal values were reached on Day 60 for both androgens. Plasma testosterone and androstenedione concentrations then regularly decreased to Month 6, by 43 % ($P < 0.001$) and 72 % ($0.01 < P < 0.001$) respectively. In old animals testosterone decreased by 65 % ($P < 0.001$) between Months 24 and 35, while androstenedione decreased by 53 % ($0.01 < P < 0.02$) between Months 20 and 24, then increased by 28 % ($P < 0.001$) between Months 24 and 35.

The ratio of plasma androstenedione:testosterone varied between birth and death, being < 1 in early life (to Day 6), > 1 from Day 7 to weaning, always < 1 in the young and the adult, and then > 1 in the old animals.

Changes in the concentrations of testosterone and androstenedione in the testis

These are shown in Text-fig. 3. There was little change for the first 6 days of life, but there was a significant ($P < 0.001$) increase between Days 6 and 18. The ratio of androstenedione:testosterone remained stable and close to 1 between birth and weaning. From Days 20 to 90, both androgens showed a second and very rapid rise of 918 % ($P < 0.001$) for testosterone and of 355 % ($P < 0.001$) for androstenedione. Between Months 3 and 6 a fall of 43 % ($P < 0.001$) occurred for testosterone and of 46 % for androstenedione ($0.01 < P < 0.02$). Testosterone concentrations gradually fell between Month 22 and 35; the decrease was 35 % ($0.02 < P < 0.05$) at Month 28 and 50 % ($P < 0.001$) at Month 35. A 50 % drop ($P < 0.001$) in androstenedione level was reached by Month 24 and the values then remained steady. The androstenedione : testosterone ratio remained < 0.4 between Day 30 and Month 35.

Discussion

This study of plasma and testicular concentrations of testosterone and androstenedione in the guinea-pig from birth to death permits definition of four main periods during the life of the species with regard to androgen secretion. (1) The neonatal period occurs from birth to Day 16; plasma and testicular androgens are relatively low but there is a transient peak in plasma testosterone on Days 2 and 3. (2) The pubertal period occurs from Day 16 to 90. It is characterized by a quick rise in plasma and testicular testosterone, starting on Day 16. Hormonal puberty can be considered as being reached on Day 50 and corresponds with the appearance of the first spermatozoa. The rapid increase in the seminal vesicle weight bears witness to the response of the accessory sex glands to androgens; there is, however, a reduction in the androstenedione:testosterone ratio in the testis as early as Day 18, which is due to a faster increase in testosterone production. The postpubertal period lasts from Day 50 to Day 90; during this stage plasma and testis testosterone concentrations remain stable and very high; the testis and genital tract continue to develop. (3) The period of adulthood begins between Months 3 and 6 and lasts until Month 24. The stable androgen concentrations in the plasma and testis between Months 6 and 24 can be considered as the basic values of the adult and are lower than those observed at puberty. (4) The period of senescence is that occurring when male guinea-pigs live beyond Month 24. At 28 months of age the testicular and plasma testosterone levels are very low and the drop is concomitant with a decrease in the testicular and seminal vesicle weight, a degeneration of the seminiferous epithelium and an involution of the seminal vesicle epithelium.

These four periods, based on the androgen concentrations in the plasma and testis, need to be more precisely determined by study of the metabolic clearance and testosterone rates and by corre-

lation with the different stages of spermatogenesis, as Courot (1971) has done for the lamb. In a preliminary study on the peripheral metabolism of testosterone in the guinea-pig we have shown that the changes of testosterone production rate are parallel with those of plasma testosterone concentrations (Jarrige, Lac, Robert & Rigaudière, 1975).

The existence of a peak in plasma testosterone during Days 1–3 in the guinea-pig was shown by Pelardy, Rigaudière & Delost (1972) and correlates with high values of plasma LH (Donovan *et al.*, 1975). A similar peak has been found in man between Days 30 and 60 (Forest, Cathiard, Bourgeois & Genoud, 1974) and is associated with high LH levels (Faiman, Reyes & Winter, 1974). However, as no parallel increase of testicular testosterone was observed in the guinea-pig, the early effect of gonadotrophins will have to be confirmed. It is possible that the adrenal gland is involved since an androstenedione peak has been observed in this gland in guinea-pigs at 12 hr of age (Pelardy & Delost, 1975). Although Donovan *et al.* (1975) did not observe a rise in plasma LH between Days 15 and 45 in the guinea-pig, it seems likely that there is gonadotrophic stimulation at this period, because of the increase in the concentrations of testicular testosterone between Days 16 and 50. A rise in plasma LH concentrations has been reported at the time of puberty in man (Wieland, Chen, Zorn & Hallberg, 1971; August *et al.*, 1972) and rat (Swerdlow, Walsh, Jacobs & Odell, 1971; Miyachi, Nieschlag & Lipsett, 1973; Döhler & Wuttke, 1975). Since the hormonal activity of the guinea-pig testis starts well before spermiogenesis and since the testis contains large amounts of testosterone and androstenedione at puberty, even before reaching its full development, androgens or their reduced metabolites may perhaps be involved in the initiation of spermatogenesis in this species. This is not so in the lamb, in which growth of the testis directly depends upon gonadotrophic stimulation (Courot, 1971).

Higher plasma testosterone concentrations in pubertal than in adult guinea-pigs are also found in the mouse and rat, but not in ram, monkey, pig and man in which the highest values are only reached in the adult (Table 1). Our finding of a relatively low concentration of plasma testosterone in adult guinea-pigs confirms those of Rivarola *et al.* (1968) and Resko (1970a). These concentrations, like those of the dog (Tremblay *et al.*, 1972) and rat (Table 1), may be low compared with those in rabbit (Mahoudeau, Corvol & Bricaire, 1973), bull (Katongole, 1971), ram, monkey and man (Table 1) and may be related to the presence of testosterone-binding globulin (Corvol & Bardin, 1973). This protein has been identified in rabbit, ram, bull, monkey and man, but not in guinea-pig, rat and dog. Therefore, testosterone, which is not protected from hepatic catabolism by a specific binding protein, would be metabolized quickly and this would explain its low concentration in plasma. The high testosterone metabolic clearance rate in the adult guinea-pig (Jarrige *et al.*, 1975) would support this hypothesis. However, before reaching definite conclusions about the relationship between the concentrations of testosterone and age it is necessary to check the influence of the adult's sexual behaviour on its own testosterone concentrations. Indeed, our experimental adults were breeders, whereas the pubescent guinea-pigs were virgin. Preliminary study showed that in virgin adults plasma testosterone concentrations are as high as during puberty.

The drop in plasma testosterone concentration observed in the ageing guinea-pig is similar to that observed in man (Table 1) and the endocrine secretion of the testis seems to be affected before spermatogenesis. Testicular involution may be due to a failure of gonadotrophic stimulation or to a drop in the receptivity of the seminiferous tubule and Leydig tissue to gonadotrophins. The rise in plasma androstenedione established in the male guinea-pig at the end of life might be due to a change in the rate of conversion of androstenedione to testosterone in the testis or in the liver, linked perhaps with a reduction of the 17β -hydroxysteroid dehydrogenase activity or to an increased androstenedione secretion by the adrenals. Although in some species, eg. the rat (Shapiro & Leatham, 1970), the adrenal weight does not change during senescence, in the guinea-pig the adrenals continue to increase in weight between Months 9 and 35 and plasma cortisol is very high in the old animals (unpublished observations), suggesting hyperactivity of the adrenal in the last month of life.

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Table 1. Comparative data for testosterone levels in the plasma of various species at different periods of androgenic activity

Plasma testosterone concentrations (ng/10 ml)						
Species	Infancy (immature) animals	Puberty	Adulthood	Senescence	Assay procedures*	References
Guinea-pig	8 N.D.† 1	61 5	36 26 31	<22	GLC GLC DID	Present results Resko, 1970a Rivarola <i>et al.</i> , 1968
Rat	1-2‡ 8 5-11	9-77 57	20-42 20		GLC CPB RIA	Resko, Feder & Goy, 1968; Knorr <i>et al.</i> , 1970 Grota, 1971 Harris & Bartke, 1974; Döhler & Wuttke, 1975; Gupta <i>et al.</i> , 1975b
Mouse	25§ 30	95§ 50	58§ 20-208		CPB RIA GLC-CPB	McKinney & Desjardins, 1973 Courrot, 1974 Attai, 1970; Katongole, 1971
Pig	9	20-80	60-100		RIA	Meusy-Delsolle, 1975
Rhesus monkey	2.5-5	8	40-89		GLC	Resko, 1967, 1970b; Snipes, Forest & Migson, 1969
Man	4	7-46	65 63		DID GLC	Frasier <i>et al.</i> , 1969 Vermeulen <i>et al.</i> , 1972
	2	7-48	54-62	58-24 46	CPB	August <i>et al.</i> , 1972; Pirke & Doerr, 1973
	1-3	3-48	43-74	61-27	RIA	Hasan, Weber, Neumann & Friedreich, 1973; Nieschlag <i>et al.</i> , 1972; Knorr <i>et al.</i> , 1974; Mazzi, Riva & Bernasconi, 1974; Stearns <i>et al.</i> , 1974; Gupta, Atanasio & Raaf, 1975a; Haug, Aakvaag, Sand & Torjesen, 1974

* GLC, gas-liquid chromatography; DID, double isotope derivative method; CPB, competitive protein binding; RIA, radioimmunological.

† Not detectable.

‡ The range of values given in the various references.

§ Total plasma androgens.

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