Small male body size in garter snake depends on testes

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CREWS, DAVID, MAIRE ANNE DIAMOND, JOAN WHITTIER, AND ROBERT MASON. *Small male body size in garter snake depends on testes.* Am. J. Physiol. 249 (Regulatory Integrative Comp. Physiol. 18): R62–R66, 1985.—In the red-sided garter snake (*Thamnophis sirtalis parietalis*) adult females are larger than adult males; this difference is apparent within 3 wk of birth, a time coinciding with high circulating levels of androgens. To study the ontogeny and regulation of this sexual dimorphism, male neonates were either castrated, castrated and given Silastic capsules containing testosterone or estradiol, or given a sham operation at 8, 9, or 10 wk of age. Female neonates were either given a Silastic capsule containing testosterone or dihydrotestosterone or given a sham operation at 8, 9, 10, or 14 wk of age. The sex difference in body size and growth rate in neonates was abolished by castration; the pattern of growth of castrated males was similar to sham-operated females. Androgens in the amounts administered failed to reverse the effects of castration, because castrated male and female neonates receiving exogenous androgens grew at the same rate as did sham-operated females. Males castrated as adults grow larger than adult males given a sham operation, indicating the inhibitory role of the testes on body size exists after sexual maturity. Treatment of adult males with testosterone, however, prevented the increase in body size after castration, suggesting that the mechanism regulating weight gain in the garter snake depends on gonadal androgen.

growth; sexual dimorphism; reptile; development; hormones

SEX DIFFERENCES IN BODY SIZE are common in animals. In some species the male is larger, whereas in others the female is larger (9, 13, 15, 18, 19, 24, 30, 32). In those animals studied, gonadal hormones early in life are important in the regulation of this sexual dimorphism in body growth. Treatment of newborn female rats and chickens with exogenous androgens results in a pattern of growth resembling that of males, whereas castration of newborn males results in a pattern of growth resembling that of females (31, 32). Androgen treatment of sexually inactive or castrated adult males leads to changes in mass and morphology as a result of the anabolic effects of androgens and the direct action of sex steroid hormones on body weight regulating mechanisms in the central nervous system (31).

There is a marked sexual dimorphism in both body mass and body length in the red-sided garter snake (*Thamnophis sirtalis parietalis*) (12). Nonpregnant females are more than three times heavier than males; adult females (n = 108) average 100.5 ± 26.7 (SD) g in body mass (BM) and 61.3 ± 4.6 cm in snout-to-vent length (SVL), whereas adult males (n = 130) average 32.6 ± 10.9 g and 46.0 ± 5.2 cm (J. Whittier et al., unpublished data). The purpose of this paper is threefold: *1*) to establish and quantify the ontogeny of the sexual dimorphism in body size in the red-sided garter snake, *2*) to determine if this sex difference in body size is influenced by removal of the gonads early in life or after sexual maturity, and *3*) to determine if the sex difference in the pattern of growth is influenced by the administration of sex steroid hormones. We report that sex difference in body size is apparent by the 3rd wk of life at a time coinciding with high circulating levels of androgens. Further, this sex difference in both neonates and adults is abolished by castration. The growth pattern of castrated male neonates is similar to sham-operated female neonates. Similarly, males castrated as adults grow larger than intact males. Administration of testosterone to adult males prevents the increase in body size after castration.

MATERIALS AND METHODS

Animals. All neonates were born in the laboratory during 1982. Shortly after birth the neonates were sexed by gently probing for the hemipenes; males and females were placed in separate cages. Sex was later confirmed surgically when the neonates were assigned randomly to a treatment group; at this time each individual was given a unique clipping pattern of the ventral scales (5).

Adult males (41–56 cm long) were collected near Narcisse Community Pasture, Manitoba, Canada, during spring emergence, 10–30 May 1983.

Housing and maintenance. Hormonally manipulated neonates were housed with sham-operated neonates in a 29-gal aquarium measuring 75 × 46 × 32 cm. The substrate consisted of pine shavings. The aquaria were situated in an environmentally controlled room programmed to provide a 14:10 light-dark (LD) photic cycle and a corresponding daily thermal cycle of 26:16°C; relative humidity was maintained at 40%.

Adult males were housed similarly; however, adults were placed in artificial hibernation conditions (0:24 LD, constant 4°C) for 17 wk during November 1983–February 1984.

Animals were fed a diet of canned fish supplemented with Petco Vitamin-Mineral Powder plus thiamine three times each week. The amount of food consumed by each individual was not recorded. Water was available at all times for drinking and soaking.

Surgery. All neonatal snakes were subjected to cold anesthesia. Adults were given methohexital sodium

(0.015 μg/g BM). Gonadectomies were performed using the technique of Camazine et al. (4). Other animals were subjected to a sham operation in which the gonads were manipulated but not removed.

Hormonal manipulation. Whole clutches of neonates (n = 8: 86 males, 116 females) were manipulated at the same time (6); representatives of each clutch were included in the different treatment groups although the group assignment was random. Male neonates were either castrated, castrated and given a Silastic capsule containing crystalline-free testosterone or estradiol-17β, or given a sham operation at 8, 9, or 10 wk of age. Because more female neonates were available, experimental groups were either given a Silastic capsule containing crystalline-free testosterone or free dihydrotestosterone or given a sham operation at 8, 9, 10, or 14 wk. Capsules measuring 0.6 mm ID × 1.2 mm OD × 5 mm length were placed in the peritoneal cavity. Control implants consisted of empty capsules. Blood levels of four sex steroid hormones were determined by radioimmunoassay in intact and testosterone-treated male neonates.

Testosterone was administered to adult snakes via subcutaneous implantation of Silastic capsules measuring 1.5 mm ID × 2.0 mm OD × 20 mm length. A capsule of this size will maintain circulating levels of testosterone (mean 7.47 ng/ml) within the physiological range observed in intact red-sided garter snakes at the height of testicular recrudescence (mean 6.47 ng/ml) (5).

Measures of body growth. Neonates were weighed (in g) and their snout-to-vent length (in cm) measured weekly. An index of body size (BM/SVL × 100) was also calculated for each individual. After *week 10* the length and mass of individuals, rather than of clutches, were monitored. In a long-term study with adult snakes, males were weighed and measured when captured in the field; final BM and SVL were determined 14 mo later, after two summer-like seasons in captivity. In a short-term study with adult males, animals were assigned to experimental groups in mid-July; final determinations of BM and length were made 7 wk later in September.

Care was taken to weigh animals at least 2 days after their last feeding to factor out the mass of food ingested. All measures were taken without knowledge of treatment group.

Statistical analysis. Repeated-measures ANOVA (BMDP statistical program using Greenhouse-Geisser probabilities) was used to determine significant differences in the slope of log-transformed BM and SVL curves and significant effects of sex (8). Post-treatment effects and planned comparisons were analyzed at the end of the experiment (*wk 18* for neonates; *wk 7* for adults) by one-way analysis of treatment on log BM with log SVL as a covariant. Differences in body growth index between castrated adult males and sham-operated adult males after 14 mo were determined using a one-tailed Student's t test. Nonparametric statistics also were used where appropriate.

RESULTS

Circulating levels of sex steroid hormones in neonatal garter snakes. Male neonates had uniformly low circulating levels of progesterone, dihydrotestosterone, and estradiol in the 1st wk of life (Table 1); testosterone levels were above 1 ng/ml in five of the eight intact male neonates sampled. This pattern was repeated in 2- to 3-wk male neonates with testosterone levels being unusually high. Female neonates maintained the low levels of progesterone, testosterone, and dihydrotestosterone during the first 3 wk of life; more than half of the females had a circulating concentration of estradiol in excess of 16 ng/ml.

Sexual dimorphism in BM and SVL in neonatal garter snakes. With the exception of *wk 1, 2,* and *10–12*, the mean BM and SVL of sham male and female neonates was separated by more than two SEs (Fig. 1). The present study and unpublished data reveal that at 3 wk females weigh an average of 1.78 ± 0.07 (SE) g (n = 185), whereas males weigh an average of 1.50 ± 0.05 (SE) g (n = 114). The slope of the BM and SVL curves of males and females did not differ in the first 9 wk (log BM: males 0.0508, females 0.0605, $P > 0.05$; log SVL: males 0.0137, females 0.0183, $P > 0.05$). However, the slopes of the log BM and log SVL curves of males and females differed after 10 wk (log BM: males 0.0065, females 0.0240 F = 8.20, $P < 0.0001$; log SVL: males 0.0045, females 0.0137, F = 29.10, $P < 0.0001$).

Effect of age at manipulation on BM of neonatal garter snakes. The age of the individual at treatment had no apparent effect on growth. Neonates treated at 8, 9, 10, and 14 wk showed similar patterns in body growth (Means Test followed by Fishers Exact Probability, P = 0.05). Therefore in all determinations of hormone treatment effects these age groups were combined.

Effect of castration and hormone treatment on BM and SVL of neonatal garter snakes. There were significant differences in growth curves of the snakes (log BM: F = 3.52, $P < 0.001$; log SVL: F = 6.38, $P < 0.001$) (Fig. 2). Castration of male neonates resulted in a pattern of

TABLE 1. *Circulating levels of sex steroid hormone in intact and hormonally manipulated neonatal male and female red-sided garter snakes*

Group	n	Progesterone	Testosterone	Dihydrotestosterone	Estradiol
Intact male (4–5 day)	8	0.12 ± 0.04	1.03 ± 0.14	0.17 ± 0.09	0.22 ± 0.10
Intact male (13–20 day)	3	0.30 (0.07–0.65)	64.46 (24.54–122.49)	2.76 (0.49–5.13)	0.46 (0.16–0.91)
Castrate + testosterone	5	0.19 ± 0.13	16.85 ± 2.83	4.86 ± 1.91	0.12 ± 0.02
Intact female (3–5 day)	11	0.20 ± 0.02	0.56 (0.09)	0.09 (0.02)	15.94 (4.68)
Intact female (11–18 day)	3	0.37 (0.14–0.71)	1.28 (0.68–1.93)	0.10 (0.04 0.21)	17.07 (0.20–34.38)

Values are means ± SE expressed in ng/ml. Range in parentheses for small sample sizes.

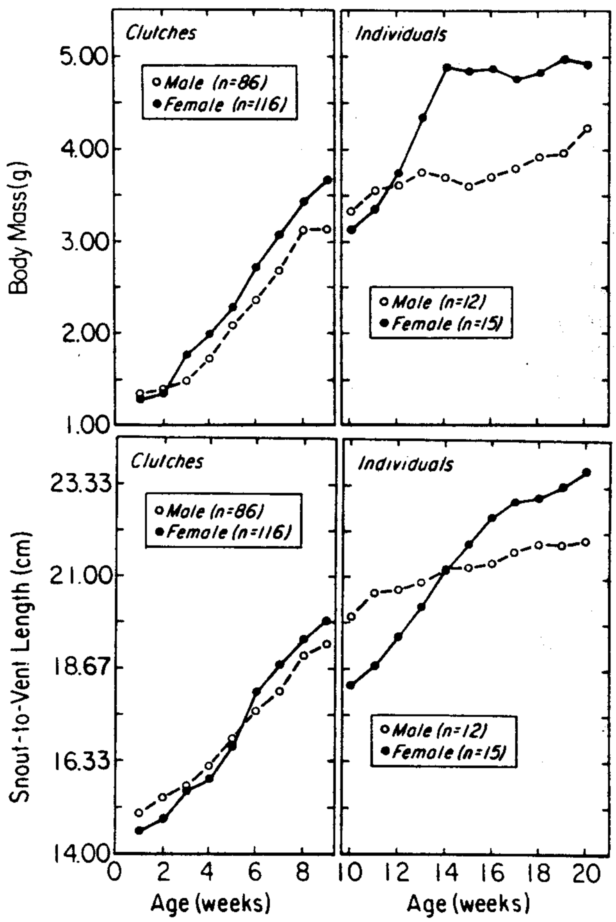

FIG. 1. Growth in newborn red-sided garter snakes. For purposes of this analysis, body size of neonates through their first 9 wk are presented as means for whole clutches of animals (8 clutches consisting of 86 males and 116 females), whereas for *wk 10–19* these data represent individuals (12 males, 15 females). Difference in body mass and length between data for clutch and data for individuals is due to a sampling artifact that resulted from random selection of individuals.

growth that did not differ significantly from that of sham-operated females (T value = 0.858, $P = 0.40$) (Fig. 3). The growth of castrated male neonates treated with exogenous testosterone was not different statistically from that of untreated castrates ($T = -0.106$, $P = 0.92$) or sham-operated females ($T = 0.880$, $P = 0.38$). The growth of sham-operated females treated with testosterone or dihydrotestosterone was not different from that of untreated females ($T = -0.866$, $P = 0.39$). All but two of the 14 castrated males treated with estradiol died within 10 wk of hormone administration; nine snakes had died by *wk 7* when the capsules were removed from the remaining neonates. Both surviving estrogen-treated castrates appeared healthy and grew at a rate similar to that of sham-operated females.

Effect of castration and hormone treatment on BM and SVL of adult garter snakes. The increase in body size of males castrated as adults ($n = 8$) and maintained in captivity for 14 mo was greater than that of sham-operated adult males ($n = 6$) (castrates 34.20 ± 6.51 (SE) g vs. shams 18.46 ± 4.94, $t = 1.802$, 12 df, $P < 0.05$). In the short-term study there were significant differences in the weight gain in the snakes (log BM: $F = 6.504$, $P = 0.002$; log SVL: $F = 0.980$, $P < 0.05$). Castration resulted in a significant increase in BM ($T = -4.102$, $P = 0.0004$) (Table 2). Intact and castrated adult males treated with testosterone did not differ significantly ($T = 0.350$, $P = 0.729$) or from intact sham-operated males

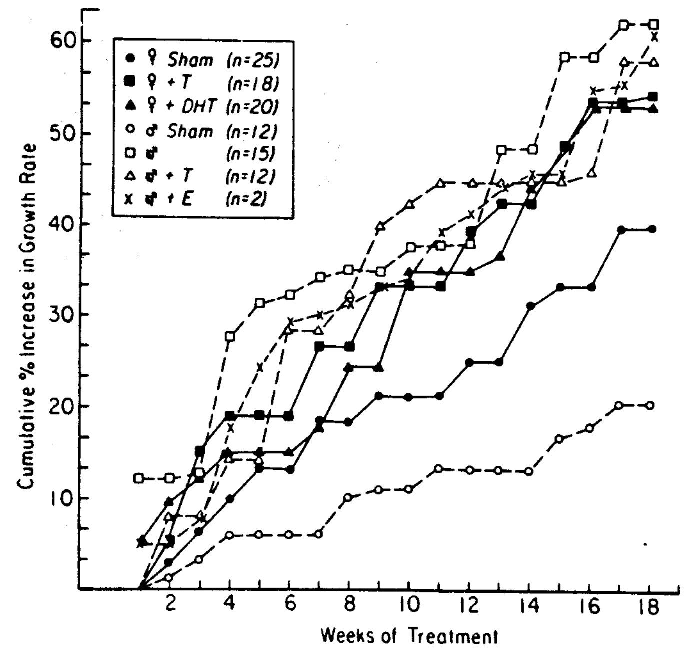

FIG. 2. Growth rate of hormonally manipulated neonatal red-sided garter snakes. Depicted is mean cumulative percent increase in weight gain each week in different treatment groups. Sample sizes shown in parentheses. T, testosterone; DHT, dihydrotestosterone; E, estrogen.

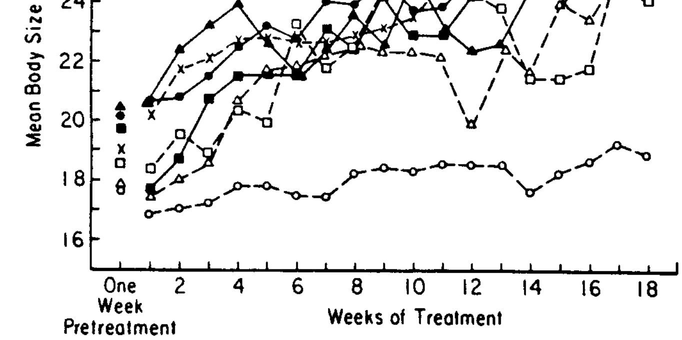

FIG. 3. Effect of gonadectomy and hormone manipulation on mean body size in neonatal male (*dashed lines*) and female (*solid lines*) red-sided garter snakes. Although data were analyzed as body mass (BM) with length (SVL) as a covariant (see text), ratio of mass to length is presented here. Sample sizes shown are in parentheses. T, testosterone; DHT, dihydrotestosterone; E, estrogen.

TABLE 2. *Changes in body mass and length in adult male red-sided garter snakes hormonally manipulated during height of testicular recrudescence*

Group	*n*	Beginning (20 Jul)	End (16 Sep)	% Change
		Mass, g		
Sham	6	30.20±2.44	29.70±2.09	−1.22±2.28
Sham + testosterone	10	28.36±1.92	29.84±2.11	6.05±4.27
Castrate	6	32.18±2.13	40.00±1.64	26.51±2.10
Castrate + testosterone	9	29.86±2.73	28.44±2.47	−4.09±2.81
		Mean length, cm		
Sham	6	49.08±1.39	49.08±1.39	0.00±0.00
Sham + testosterone	10	46.90±1.08	46.95±1.24	0.08±0.96
Castrate	6	47.17±0.79	48.58±0.96	3.03±1.43
Castrate + testosterone	9	46.39±1.05	46.17±1.28	−0.56±0.69

Values are means ± SE.

($T = -1.242$, $P = 0.225$ and $T = -0.901$, $P = 0.376$) (Table 2).

DISCUSSION

A clear sex difference was found in both body mass and snout-vent length in neonatal red-sided garter snakes. Females were significantly heavier than males by the 3rd wk of postnatal life, and this difference persisted. This sudden difference in size between male and female neonates coincided with unusually high levels of testosterone in male neonates. Indeed the levels observed in 2- to 3-wk neonatal males were approximately 10 times that observed in adult males during testicular recrudescence. Female neonates, however, maintained uniformly high levels of estradiol and low levels of androgens during the same time period.

Sexual dimorphisms in body mass and skeletal growth have been extensively studied in domesticated and laboratory-bred animals. With relatively few exceptions, the male is the larger sex in mammals (15, 18, 19, 30). In those species examined in this regard, weight gain is regulated by gonadal sex steroid hormones acting both early in life and in adulthood (31). For example, in rats gonadal hormones secreted shortly after birth alter adult body mass (2, 3, 29); gonadectomy immediately after birth abolishes adult sex differences in body mass. Further, a single injection of testosterone given to newborn ovariectomized females significantly increases adult body mass (3, 25), indicating that it is testicular androgens, not ovarian hormones, that are responsible for the sex difference in body mass in rats. In adult rats, castration leads to a decrease in mass, whereas low doses of exogenous testosterone reinstate the pattern of weight gain characteristic of intact males (10, 15, 20); high doses of testosterone have the opposite effect on weight gain, this depressive effect apparently being due to the aromatization of androgen to estrogen (10, 21).

The present study suggests that the marked sexual dimorphism in body size in the red-sided garter snake is likely due to gonadal androgens. The sex difference in body size in neonatal garter snakes was abolished by castration; castrated neonates gained mass at a rate remarkably similar to that of sham-operated female neonates. Since the concentrations of sex steroid hormones in the circulation produced by the Silastic capsules were well below the levels observed in 2- to 3-wk neonatal males, it is probable that the lack of effect of exogenous androgen treatment on body size in the neonates was due to insufficient amounts of androgen. However, body growth may be independent of sex hormone control early in life. In domesticated fowl (22) and turkeys (14), the circulating level of growth hormone is significantly higher before puberty than after puberty. This maturational decline in the plasma concentration of growth hormone is not influenced by castration or sex steroid hormone treatment (23). Further work is required to determine if body growth in the red-sided garter snake can be influenced by sex steroid hormones during development.

Males castrated as adults also increased in body mass and length. However, androgen administered in physiological amounts to castrated adult males prevented this increase. This demonstrates that gonadal androgen plays an inhibitory role in the maintenance as well as in the development of the sexual dimorphism in body size in the red-sided garter snake. Thus androgens do not appear to have an anabolic effect on growth in garter snakes.

The regulation of body mass in the Syrian hamster (*Mesocricetus auratus*) appears to be similar in some respects to the garter snake. In the Syrian hamster the female is the larger sex. Castration of the male results in a significant increase in weight gain, with castrated males eventually becoming indistinguishable from females in size (16, 27, 28, 34). Further, treatment of castrated adult male hamsters with testosterone propionate depresses body mass (27, 34). Syrian hamsters are unlike garter snakes, however, in that treatment of neonatal females with androgen reduces adult body mass (11).

In snakes, and in reptiles in general, growth is indeterminate (17), although the rate of growth slows with age (1). In those reptile species in which the male is the larger sex, male juveniles grow faster than females and vice versa in those species in which the female is the larger sex; in those species in which the sexes are similar in adult size, there is no dimorphism in the rate of growth (1). Investigation of a reptile species in which the male is the larger sex would be instructive. If the control of weight gain in such species is similar to that of rats (31), mice (33), and guinea pigs (7, 26), this would suggest that the pattern of sexual dimorphism in size in the adult reflects alternative size-controlling mechanisms.

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