The effect of daily evening isoproterenol administration on reproductive organ growth in male rats treated neonatally with testosterone propionate

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Summary. Daily evening injections of isoproterenol extended the period of high pineal N-acetyltransferase activity and retarded the growth of testes, seminal vesicles and prostate in rats treated neonatally with testosterone propionate.

Although the laboratory rat is only marginally photoperiodic[2], either neonatal treatment with testosterone propionate (TP)[3,4], olfactory bulbectomy[5] or undernutrition[6] bring its gonadal development under photoperiodic control. Sensitized rats kept for 6 weeks under photoperiods shorter than 10 h of light per day have smaller testes, seminal vesicles and ventral prostate than animals kept under longer photoperiods[7,8]. The information about the daylength is probably transduced by the pineal gland, as the inhibitory effect of short photoperiods is prevented by pinealectomy[6-8]. The rhythm of the pineal hormone melatonin is driven by the rhythm of pineal N-acetyltransferase (NAT) activity[9,10]. The period of high NAT activity is more than 1 h longer in rats kept under a 8-h light, 16-h dark (LD 8:16) lighting schedule than in those kept under LD 12:12[7]. Daily evening injections of melatonin decreased within 5 weeks the reproductive organ weights in anosmic or underfed rats kept under LD 14:10[11,12].

Both these observations suggest that extension of the period when melatonin is increased may inhibit the growth of reproductive organs. However, the quantity of melatonin given to rats was about 100 times greater than the quantity synthesized by the in situ pineal gland throughout the 24-h period[13,14]. Moreover, the route of melatonin administration was not natural. Therefore we decided to prolong the endogenous production of melatonin. It is well known that the β-agonist isoproterenol induces the increase of NAT activity[15] and consequently of pineal melatonin[10]. To induce precocious evening increase of melatonin synthesis we administered isoproterenol to TP-treated rats kept under LD 12:12 daily before lights off. After 6 weeks of the treatment we determined the weights of reproductive organs and NAT rhythm.

Materials and methods. Wistar male rats were maintained from birth under LD 12:12 (lights on from 06.00 to 18.00 h) at a temperature of 23±2 °C and fed ad libitum. 3-day-old rats were either injected s.c. with 1 mg of testosterone propionate in 0.1 ml of olive oil or left intact. Pinealectomy was performed at 28 days of age by the method of Hoffmann and Reiter[16]. Isoproterenol sulfate in 0.5 ml saline was injected s.c., daily from 35 to 75 days of age (except on days 43, 44, 51, 57, 58, 71 and 72 of age) at 17.40–18.00 h. The dose of 0.2 mg isoproterenol/kg b.wt increases NAT activity in 55-day-old rats; however, about a 10 times greater dosage is necessary in younger (40-day-old) rats[17]. Therefore we injected 2 mg/kg b.wt daily from 35 to 52

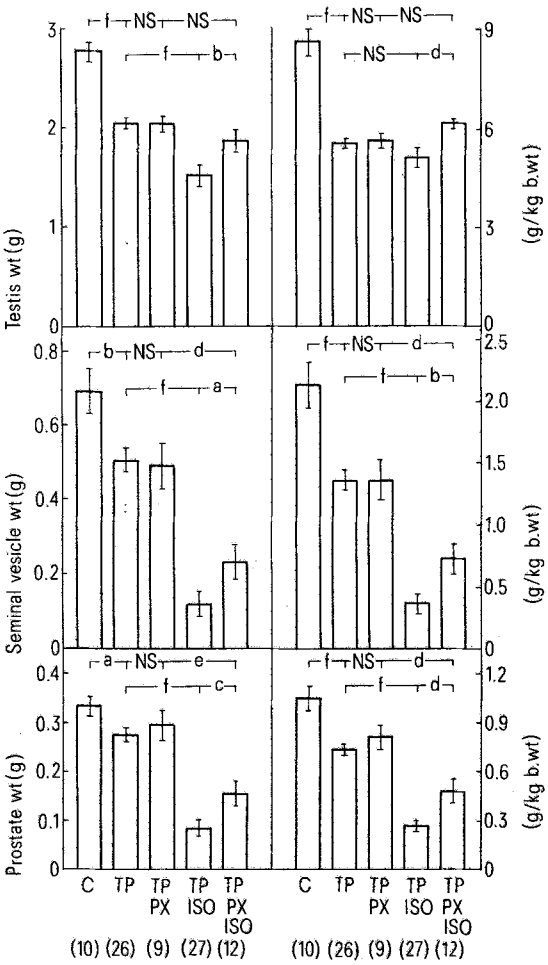

Figure 1. The effect of isoproterenol (ISO) injected for 6 weeks before lights off on testicular, seminal vesicle and prostate weights in neonatally TP-treated (TP) and pinealectomized TP-treated (PX, TP) male rats. C, intact control rats; left, absolute organ weights; right, relative organ weights in kg/b.wt. Each column represents mean (± SEM); numbers in parentheses indicate numbers of rats. $^{a}p \leq 0.05$; $^{b}p \leq 0.02$; $^{c}p \leq 0.01$; $^{d}p \leq 0.005$; $^{e}p \leq 0.002$; $^{f}p \leq 0.0001$. NS, not significantly different.

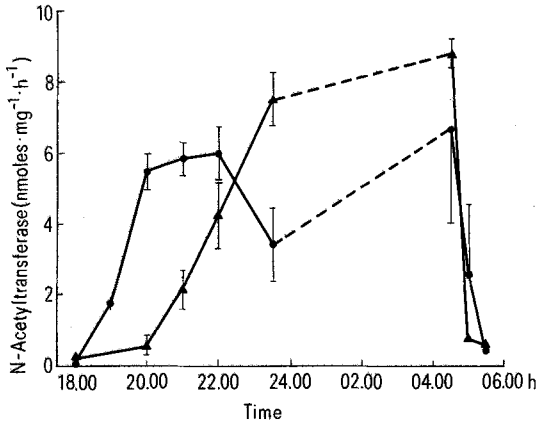

Figure 2. The effect of isoproterenol injected daily for 6 weeks at 18.00 h on the rhythm in NAT activity in neonatally TP-treated rats. ●, isoprotenerol treated rats; ▲ untreated controls. Each point represents the mean (± SEM) from 3 animals. Where SEM are omitted, they were less than diameters of the points.

days of age and 0.2 mg/kg b.wt from 53 to 75 days of age. All rats were rapidly killed at 75 days of age, in darkness under dim red light. Testes, seminal vesicles and ventral prostates were dissected and weighed. Pineals were dissected within 5 min, weighed and stored in a Petri dish on solid CO_2. NAT activity was determined within 48 h by a modification[18] of the method of Deguchi and Axelrod[19]. Differences between means were analyzed for significance using Student's t-test.

Results and discussion. As previously reported[3], neonatal TP-treatment decreased the weights of testes, seminal vesicles and prostate (fig. 1). Pinealectomy (PINX) did not affect the weights of reproductive organs in TP-treated rats kept under LD 12:12. Isoproterenol administrations lowered the weights of seminal vesicles and prostate in both intact and PINX TP-treated rats, but the testis weights only in intact animals. Isoproterenol treatment decreased the weights of reproductive organs significantly more in intact than in pinealectomized TP-treated rats. Part of the antigonadal effect of isoproterenol is thus mediated by the pineal gland.

Isoproterenol induced precocious increase of NAT activity, but it did not affect the time of spontaneous morning decline (fig. 2). The NAT activity was thus elevated above 3 nmoles $\cdot$ $mg^{-1} \cdot h^{-1}$, i.e. above the value which already gives almost maximal melatonin production[10] for 7.5 h in controls and for 9.6 h in isoproterenol treated rats. Isoproterenol treatment thus depressed the weights of reproductive organs in TP-treated rats partly via the pineal and concomitantly prolonged the period of high NAT activity. This observation supports the idea that extension of the period of high melatonin production may inhibit the growth of reproductive organs in rats treated neonatally with testosterone propionate.

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Chemical stimuli eliciting courtship by males in *Drosophila melanogaster*[1]

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Summary. By progressive removal of various sense organs, and testing for inter-male courtship in the absence or presence of females, it is demonstrated that *D. melanogaster* males require close-range, probably contact, chemical stimuli for the initiation of courtship.

Volatile chemicals have been implicated in the initiation of courtship by males in *Drosophila melanogaster*[3-6]. Removal of the antennae, which have long been considered to be the major olfactory receptors, does not, however, prevent males from courting[7,8]. Similarly, although contact chemicals received by the male when tapping the female with his foretarsi appear to be important[8,9], foretarsiless males court readily[8]. Other organs which may receive volatile and/or contact chemicals are the proboscis and the palps[9], and the aristae[10]. To resolve the relative importance of these stimuli in the initiation of courtship, and which organs receive them, males in which various combinations of these organs had been removed were tested for their propensity to court females or other males. Also, propensity for intermale courtship was tested in the absence or presence of physically distanced females to test the importance of volatile chemicals.

Materials and methods. 4-day-old Canton-S males were collected after 24 h from standard cultures, kept isosexually 10 per vial, and then singly for 20–24 h before testing. Virgin females were collected after 12 h, kept 10 per vial, and used after a further 12–24 h. The males were operated on under ether anesthesia 2 h before testing. Aristae, foretarsi (plus or minus the basitarsi which bear the sex-combs) and proboscides were snipped off with fine scissors, and palps and antennae were plucked off with forceps. Only the 3rd segment of the antenna, the funiculus, which bears the arista and the olfactory receptors[10], was removed. Some observations were conducted in red light in a photographic darkroom to remove visual stimuli. Single pairs were observed for 10 min with a binocular dissecting microscope in either side of a standard perspex observation chamber (22 mm diameter; 8 mm deep) with a double-layer nylon gauze partition. This allowed simultaneous observation of 2 pairs, and the gauze provided better purchase for the foretarsiless males. Whether the male courted, as indicated by wing extension and vibration, was noted. Although courtship is not all or none[11], quantitative