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Exposure to Early Androgen Attenuates Androgen-Induced Pup-Killing in Male and Female Mice[1]

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Adult virgin female mice administered testosterone propionate (TP) on Day 0, 4, or 8 of life require a longer TP exposure period in adulthood to induce pup-killing than do animals given TP on Day 12 or 21 or oil on Day 0. Significantly fewer males gonadectomized on Day 60 killed pups in response to TP treatment in adulthood as compared to males gonadectomized on Days 0 and 4. Day 60 gonadectomized males that did kill young required a longer exposure period to TP than did animals gonadectomized on Day 0 or 4. These data suggest that early androgen reduces the effectiveness of later androgen exposure in inducing pup-killing.

Testosterone (T) has the capacity to induce intraspecific killing of young. Fuller *et al.* (1970) reported that the administration of T to pregnant Dutch-belted rabbits led to a significant increase in cannibalism of young. Pup-killing also has been exhibited by adult female rats that were administered T on Day 3, 5, 7, or 9 of life and again on Day 40 (Rosenberg and Sherman, 1974). Moreover, both pre- and postpubertal castration of male rats will reduce the incidence of pup-killing (Rosenberg, 1974).

Approximatley 35–50% of adult male Rockland–Swiss albino mice as opposed to 10% or less of adult females kill mouse young (Gandelman and vom Saal, 1975). It has been shown that between 60 and 80% of adult females will kill young following the administration of T (Davis and Gandelman, 1972; Gandelman, 1972). These data suggest that testicular androgen is responsible for the differential killing in males and females.

We recently have found that the administration of T to adult males left intact or to males gonadectomized in adulthood which had previously displayed maternal behavior will *not* induce pup-killing (Gandelman and vom Saal, 1975). However, T will induce killing when given to adult males that have been gonadectomized on the day of birth. These findings led to the hypothesis that pup-killing results when the organism is exposed to

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relatively high adult levels of T for the first time and that early exposure to T somehow inhibits the later induction of T-induced killing. Unlike females, intact adult males and males gonadectomized in adulthood already have been exposed to relatively high levels of T. Thus, the administration of T should have a minimal effect upon responsiveness of these males to the young. This explanation also accounts for the fact that males begin to kill pups spontaneously during a period of development in which plasma androgen levels increase approximately 300% (Gandelman, 1973) and that males gonadectomized at birth and exposed to T when adult will kill young (Gandelman and vom Saal, 1975).

The hypothesis used to account for the relative inability of T to induce pup-killing in adult male mice leads to the prediction that exposing females to T early in life will attenuate the later pup-killing-inducing property of this steroid. The following experiments were designed to test this prediction. In addition, an attempt was made to determine whether the effect of early T exposure is relegated to a particular stage of early development. This was assessed in both the female and the male.

EXPERIMENT 1

Method

Nine pregnant nulliparous Rockland–Swiss (R–S) albino mice that were maintained as an outbred strain in a closed colony were housed singly in 11 × 7 × 5-in. translucent cages, the floors of which were covered with pine shavings. The animals were provided with food and water in excess and kept on a 12-hr light/12-hr dark cycle.

Each litter was reduced to six female offspring at parturition, a period referred to as Day 0. If a litter did not contain the requisite number of females, additional pups were fostered to it. Within 1 hr following reduction of the litters, each pup was ovariectomized while anesthetized with ether. The wounds were dressed with a commercially prepared plastic adhesive covering (Newskin). The pups then were placed under a heat lamp where they remained until recovering from the anesthetic (approximately 20 min), at which time the offspring of five of the litters were given one subcutaneous injection of 500 μg testosterone propionate (TP) dissolved in 0.02 ml sesame oil. The offspring of the other four litters were administered only the oil vehicle. The pups then were returned to the dam, weaned on Day 21 of life, and kept in littermate groups until Day 60.

The mice were housed singly on Day 60 and, 24 hr later, each was pretested for pup-killing behavior. Testing took place 2 to 3 hr following light onset. Three 1-day-old R–S pups were placed into each animal's cage. Fifteen minutes later the adult's behavior toward the young was recorded. An animal was scored as being maternal if the pups had been gathered together and if the female had assumed a nursing position over them. [It should be noted that mice are spontaneously maternal, display-

ing maternal activities regardless of sex and reproductive state (cf. Noirot, 1964)]. An animal was scored as a killer if it killed at least one pup. Animals that killed pups during the pretest were eliminated from the experiment.

All animals were injected subcutaneously with 500 μg of TP in 0.02 ml of sesame oil immediately following the pretest. Such injections were given daily for a maximum of 25 days. Every sixth day, 3 hr prior to an injection, the animals were tested for pup-killing using the procedure described above. Animals either were given five pup-killing tests or were terminated from further testing when pup-killing occurred.

Results

Four TP- and four oil-treated mice died between birth and weaning, leaving 26 TP- and 20 oil-treated animals. Two oil-treated and none of the TP-treated animals killed young during the pretest, i.e., prior to adult TP administration.

With few exceptions, animals either killed or were maternal toward all three pups during the tests. (In two cases, the adult killed one pup and ignored the other two.) The killing response itself typically consisted of bites to the back of the pup, after which the pup was eaten.

The results of the pup-killing tests are presented in Fig. 1. Significantly more animals that received oil on the day of birth killed pups as a

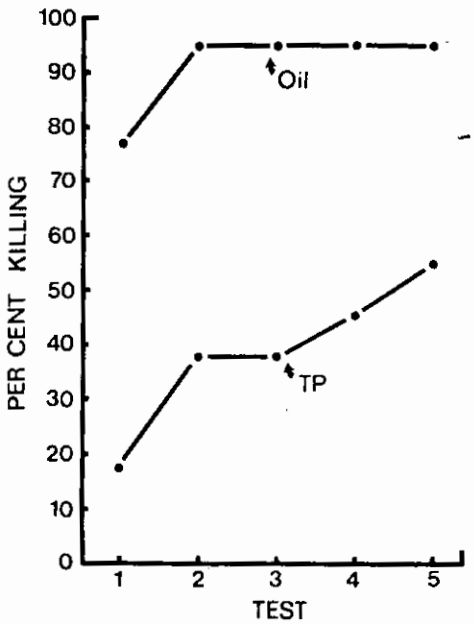

FIG. 1. The cumulative percentage of adult virgin female mice administered either oil or 500 μg of TP on the day of birth that killed pups in response to TP treatment in adulthood. Five tests for pup-killing were given, each following five daily injections of 500 μg of TP.

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consequence of TP treatment in adulthood than did mice that received TP in infancy [χ^2 (1) = 14.86, $P < 0.001$]. An overall analysis (Wilson, 1956) performed on the data from only those animals that killed pups revealed that mice administered oil killed sooner following the initiation of adult TP treatment than did animals given TP on Day 0 [χ^2 (1) = 17.57, $P < 0.001$].

The data show that the administration of TP to female mice on the day of birth attenuates the killing-inducing property of TP. Animals administered the steroid on Day 0 either did not kill in response to a maximum of 25 injections of 500 μg of TP or took longer to respond to the hormone.

The next experiment was designed to determine whether a relationship exists between the time of administration of early TP and its effectiveness in attenuating the killing-inducing property of TP. Is there a period during early development beyond which the administration of TP will not affect the ability of the steroid to induce pup-killing? In addition, the experiment ascertains whether the incidence of pup-killing will increase in Day 0 TP-treated animals if the maximum adult TP regimen is extended beyond 25 days.

EXPERIMENT 2

Method

Thirty pregnant nulliparous R–S mice were housed singly as previously described. The litters were reduced to six females at parturition and the young were ovariectomized. The litters then were assigned randomly to one of six treatment conditions with five litters in each. Initially, then, each group was composed of 30 animals. One condition consisted of the administration of 0.02 ml of oil immediately following parturition. The other conditions consisted of varying the time at which one injection of 500 μg of TP was given. The times were Days 0, 4, 8, 12, and 21. The animals were weaned on Day 21, kept in littermate groups until Day 60, and housed separately at that time.

Each animal was pretested for pup-killing 24 hr after being isolated, and those animals that killed were eliminated from the experiment. Immediately following the pretest, those mice that did not kill began to receive daily injections of 500 μg of TP and were tested every sixth day 3 hr prior to the injection. The procedure was identical to that of Experiment 1 with the exception that animals could have received a maximum of 35 injections (seven tests) rather than 25, as was the case for the first experiment.

Results

There was no significant difference between the groups with respect to the number of animals that killed during the pretest (mean = 4.8). The test data are shown in Fig. 2. The groups did not differ in the final percentage of animals that killed young. An overall analysis (Wilson, 1956) of the data for only those animals that did kill young yielded a significant Group

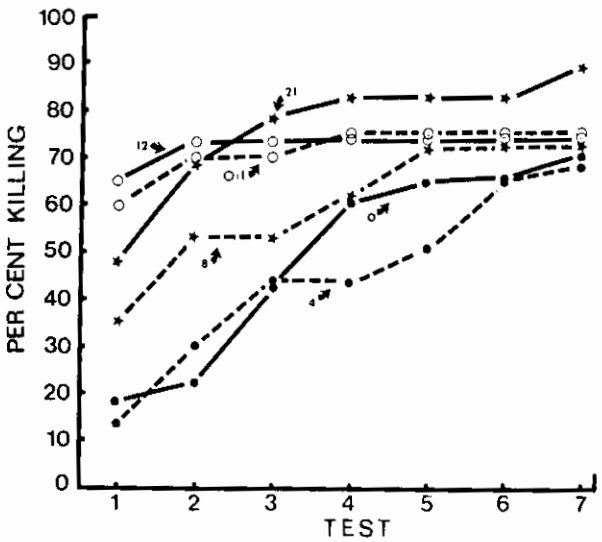

FIG. 2. The cumulative percentage of adult virgin female mice administered 500 μg of TP on Day 0, 4, 8, 12, or 21 of life or oil on Day 0 that killed pups in response to TP treatment in adulthood. Seven tests for pup-killing were given, each following five daily injections of 500 μg of TP.

Effect [χ^2 (5) = 26.49, $P < 0.001$], thus indicating that there were differences between the groups in the rapidity with which TP induced killing behavior. As the figure shows, mice that were administered oil on Day 0, TP on Day 12, or TP on Day 21 killed sooner following the initiation of adult TP treatment than did the animals of the other groups. Mice given TP on Day 0 or 4 required the longest treatment period, while animals exposed to TP on Day 8 were intermediate in their responsiveness to the adult TP regimen.

The data show that the administration of one injection of TP to females up to at least Day 4 of life significantly lengthens the duration of TP treatment required to induce pup-killing. The data also show that early TP treatment does not permanently block the killing-inducing property of 500 μg of TP given in adulthood, as was concluded from Experiment 1, if the adult TP treatment period is extended beyond 25 days. The next experiment assesses whether a relationship also exists in the male between time of early androgen exposure and the induction of pup-killing by TP treatment.

EXPERIMENT 3

Methods

The litters of 24 primiparous R–S mice, adjusted to six males soon after parturition, were assigned randomly to four groups of six litters each. The groups, composed initially of 36 animals, differed with respect to the time

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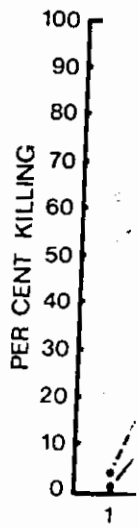

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of castration. Castrations, performed under ether anesthesia, were carried out on Day 0, 4, 10, or 60. The animals were weaned on Day 21. Those that were gonadectomized in infancy were kept in littermate groups until Day 60, at which time they were housed singly and, 24 hr later, pretested for pup-killing. Mice gonadectomized on Day 60 were kept in littermate groups until Day 90, at which time they too were isolated and pretested for killing 24 hr later. Animals that killed young during the pretest were eliminated from the experiment. Following the pretest, all animals began to receive daily injections of 500 μg of TP. They were tested for pup-killing every sixth day, 3 hr prior to being injected, and were given a maximum of seven tests (35 injections) or were terminated from testing when they killed.

Results

There was no significant difference between the groups in the number of animals that killed pups during the pretest (mean = 7.7). The test data are presented in Fig. 3. A χ^2 analysis revealed a significant difference between the groups in the percentage of animals that killed young within the test period [χ^2 (3) = 18.38, $P < 0.001$]. Individual comparisons reveal that the percentage of animals that killed pups did not differ between Groups 0 and 4, whereas both of these groups differed from Group 60 [Group 0 vs Group 60: χ^2 (1) = 12.6, $P < 0.001$; Group 4 vs Group 60: χ^2 (1) = 6.64, $P < 0.01$]. Group 10 was intermediate, not differing from either

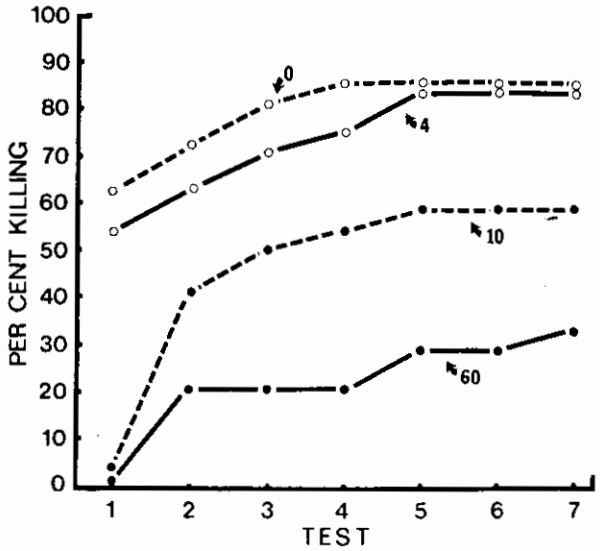

FIG. 3. The cumulative percentage of adult male mice gonadectomized on Day 0, 4, 10, or 60 of life that killed pups in response to TP treatment later in life. Seven tests for pup-killing were given, each following five daily injections of 500 μg of TP.

Group 4 or Group 60. Finally, an analysis (Wilson, 1956) based upon the data of only those animals that killed young reveals that the groups differed significantly with respect to the rapidity with which the adult TP regimen produced killing behavior [χ^2 (3) = 24.46, $P < 0.001$]. Mice gonadectomized on Days 0 and 4 killed sooner than did animals gonadectomized on Day 10. Mice gonadectomized on Day 10 fought sooner than did mice gonadectomized on Day 60.

DISCUSSION

The results of these experiments show that exposure to TP during early development attenuates the pup-killing-inducing property of this steroid when administered later in life. For the female, the attenuation took the form of a lengthening of the adult TP treatment period required to induce killing behavior. The data also show that the period during which TP exposure can affect the females' later responsiveness to the hormone extends from the time of birth to sometime prior to Day 12 of life, with TP exposure between birth and Day 4 producing the greatest amount of attenuation. It is possible, however, that this relationship found between time of early TP exposure and later responsiveness to TP is dose dependent, with the maximum effectiveness of a lower dose of TP not extending to Day 4.

The data also show that early exposure to androgen affects males as well as females. It appears that early androgen, for a significant proportion of males, can completely block responsiveness to later TP, since it was found that significantly more males gonadectomized on Days 0 and 4 killed young in response to adult TP treatment than did animals gonadectomized on Day 60. The proportion of males gonadectomized on Day 10 that killed was intermediate and did not differ from the other three groups. Thus, in males, the presence of testicular androgen sometime beyond Day 4 of life can reduce the ability of TP to induce the killing of young. In addition to the inhibiting effect in some animals on TP-induced pup-killing, early androgen exposure also lengthened the duration of the adult TP treatment period required to induce killing in the males that did kill young. Those mice gonadectomized early in life required a significantly shorter exposure period to TP than did animals gonadectomized later.

The results of this study support the explanation put forth to account for the relative inability of TP to induce pup-killing in males that do not kill spontaneously, namely, that early exposure to androgen will block TP-induced killing (Gandelman and vom Saal, 1975). Through what mechanism is the effect mediated? Generally, a consideration of the effect of early androgen exposure upon the efficacy of later androgen treatment leads to the conclusion that either no effect is produced or early androgen facilitates the effect of later androgen. For example, early androgenic stimulation does not facilitate the induction of mounting behavior by

androgen treatment in adulthood (Edwards and Burge, 1971; Whalen and Edwards, 1967). However, early androgen exposure facilitates the ability of androgen to induce intraspecific fighting behavior (Bronson and Desjardins, 1970; Edwards, 1968; Svare *et al.*, 1974). As we can see, then, the attenuating effect on pup-killing of early androgen is not analogous to the effects of early androgen on other behaviors that require androgen later in life. It is, however, akin to the effect of early androgen exposure upon the adult's responsiveness to other gonadal steroids. For example, animals exposed to androgen early in life rarely display lordosis in response to estrogen–progesterone treatment (Edwards and Burge, 1971; Grady *et al.*, 1965). Early treatment with TP also inhibits the later induction of maternal nest-building with progesterone and estrogen (Lisk *et al.*, 1973). It appears, then, that early androgen suppresses responsiveness to the sexual and maternal behavior-inducing properties of estrogen–progesterone. The present findings suggest that early androgen also suppresses responsiveness to itself with respect to the induction of pup-killing.

Another explanation to account for the effect of early androgen upon later pup-killing deals with the possibility that such exposure alters early social behavior. Since the animals were kept in like-treatment groups from birth until Day 60 of life, it is possible that differential social interaction may have mediated the effect upon pup-killing. Although this explanation may be correct, informal observations did not reveal differences in group behavior between any of the treatment groups. Nevertheless, this possibility merits further consideration.

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BEHAVIORAL BIOLOGY **20**, 261–269 (19

Influence of Adaptatio of Humans to Inc Rate with Instru

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Psychology Department, Univ

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[1] This research was supported, in p Kansas.

[2] To whom requests for reprints sho