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Effects of Ventricularly Implanted Sex Steroids on Calling and Locomotor Activity in Castrated Male Japanese Quail

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To clarify the different actions of steroid hormones on calling and locomotor activity, minute pellets of steroid hormones were stereotaxically implanted into the third ventricle of castrated Japanese quail. Testosterone (T) pellets were effective in inducing calling to about 60% of that observed in castrated quail given subcutaneous implants of T. However, implants of 5α-dihydrotestosterone (5α-DHT) were completely ineffective and effectiveness of estradiol-17β (E_2) was very slight, if any. On the other hand, E_2 and T pellets enhanced locomotor activity; E_2 was more potent than T, whereas 5α-DHT was again ineffective. Cholesterol pellets had no effects on either behavior. Daily rhythms of calling and locomotor activity were also found in birds given ventricular T implants. These results indicate that T but not E_2 is required for induction of calling and that aromatization occurs in the brain to exert enhanced locomotor activity. The results also indicate that changes in circulating T do not influence daily rhythms of calling and locomotor activity.

INTRODUCTION

It has been demonstrated repeatedly that testicular androgen is essential for stimulation and maintenance of sexual behavior in avian species (see Silver, O'Connell, and Saad, 1979). Recently, testosterone and its metabolites have been tested for their potencies to induce sexual behavior (Adkins and Nock, 1976; Adkins and Pniewski, 1978; Balthazart, Massa, and Negri-Cesi, 1979; Wada, 1982). Several investigations suggest that testosterone (T) is converted in the central nervous system to estradiol-17β (E_2) which is an active metabolite inducing sexual behavior. Balthazart *et al.* (1979) also argued that enzymatic activity of 17β-hydroxysteroid dehydrogenase in the hyperstriatum is positively correlated with behavior.

Apart from sexual behavior, there are few works on hormones and behavior using avian species. In previous papers (Wada, 1981, 1982), I showed that calling was directly dependent on T, and activity on E_2. However, there is a discrepancy among investigators as to which steroid

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is most effective in inducing vocalization in quail. Adkins and Pniewski (1978) showed that injection of 5α-dihydrotestosterone (5α-DHT) was more potent to induce calling than T in Japanese quail, but Wada (1982) could not find positive results with Silastic implantation of 5α-DHT in the same species. Deviche and Schumacher (1982) also observed calling with injection of 5α-DHT. The main differences of these investigations were the methods of observation and the ways of hormone administration. As for hormone administration, subcutaneous implantation of Silastic capsules has a drawback when different hormones are applied and compared; there are different rates of release from the Silastic capsules of the similar size (Balthazart and Hirschberg, 1979; Wada, 1982).

To avoid this drawback, one possible way is to implant steroids directly into the brain. This also assures that the implanted hormones act on the brain to induce behavior. However, few investigations have been performed on effects of implanted steroids into the brain on calling and locomotor activity. Phillips and Barfield (1977) failed to induce crowing in capons by T implantation into the midbrain vocal area (Peek and Phillips, 1971) and the preoptic area. Since there is no work which succeeds to induce calling by hormone implantation to the specific brain sites, it might be better to implant hormones into a more general site in the brain, the third ventricle.

Thus the purpose of the present experiment is (1) to test whether steroids implanted into the brain can elicit calling and enhance locomotor activity, and (2) to clarify which steroids really act on which behaviors.

MATERIALS AND METHODS

Animals. Male Japanese quail (*Coturnix coturnix japonica*) were purchased from a commercial source at the age of 3 weeks and kept in a colony under 8L16D (lights on from 0900 to 1700) for about 2 to 4 weeks. The birds were given pelletized quail food and water *ad libitum*. They were castrated before testicular development and kept under short days until the start of the experiment.

Apparatus. During the experimental period, each bird was kept in a recording cage enclosed in a light-tight box described elsewhere (Wada, 1981). Briefly, the cage, box, and electronic devices were designed to detect sounds with a frequency of 1 to 10 kHz and with a certain duration, and to detect floor deflections. Since a quail call has a frequency range of up to 6 to 7 kHz and continues for about 0.5 sec in the last note, each call (crow) induces one pulse; background noises and calls other than crows do not. Each event was counted and recorded continuously, and the cumulative numbers were printed out for every clock hour. Inside the box illumination was provided by a fluorescent lamp and a 24-hr light–dark cycle (16L8D; lights on from 0900 to 0100) was regulated by an external timer.

Experimental schedules. At the beginning of the experiment, all the

birds were implanted subcutaneously with Silastic capsules (3.18 mm o.d. and 1.57 mm i.d., 2 × 30 mm) containing crystalline T (Sigma, St. Louis, Mo.) between 1000 and 1100 to be sure that each castrated bird responded to T treatment. This size of T capsule induces calling, enhancement of locomotor activity, and growth of the cloacal gland to the level found in intact photostimulated birds (Wada, 1981). After 2 weeks, when numbers of calls and locomotor activity leveled off, the capsules were removed between 1000 and 1100. Two weeks after the capsules were removed, birds were implanted intraventricularly with a small pellet of steroid hormone. After 2 weeks, birds were killed by decapitation. During the experimental period, cloacal protrusion area was measured every 2 or 3 days with a ruler.

Implantation into the third ventricle. Implantation was carried out under pentobarbital anesthesia during 1000 to 1600. Pellets for implantation were prepared according to Hayashi (1979): Each of the steroids mentioned below and paraffin were well mixed (1:2 by weight) with a small amount of charcoal powder. The steroids tested were T, 5α-DHT, E_2, and cholesterol (Chl). A mixture was inserted between two glass plates separated by a wire with 0.5 mm diameter. With an aid of a hot plate, the mixture was spread out to make a sheet with 0.5 mm thickness. Then a pellet (0.7 × 1.0 mm high) was loaded into the tip of stainless-steel tubing (1.0 mm o.d. and 0.7 mm i.d.) by punching out the sheet twice. This stainless-steel tubing was attached to a stereotaxic apparatus modified for quail. With the aid of the apparatus, the pellet was extruded by a plunger into the third ventricle at the medial basal portion.

At the end of the experiment, birds were killed by decapitation and the brains were dissected for fixation in Bouin's solution. With usual histological procedures, each brain preparation was examined to verify the position of the implant in the third ventricle. Data were discarded when the pellet was found not to contact the ventricle.

Weights of pellets implanted were estimated by comparison with those prepared in the same way as described above. They were weighed with a microbalance to the nearest 1 μg.

Statistics. Statistical analyses were carried out by Student's t test.

RESULTS

All the castrated birds showed calling and enhanced locomotor activity at the levels found previously (Wada, 1981) after subcutaneous implantation of T capsules (Figs. 1 and 2). Removal of T capsules abolished calling and reduced locomotor activity to castration levels after a week or so. Two weeks after removal of T capsules, cholesterol, T, E_2, or 5α-DHT were implanted. Different steroids implanted into the third ventricle caused different results both in calling and locomotor activity.

Table 1 shows weights of micropellets prepared in the same manner

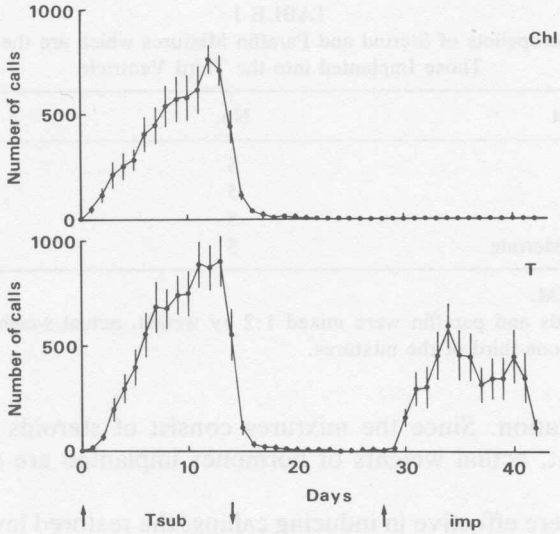

FIG. 1. Changes in numbers of calls during subcutaneous testosterone implantation (Tsub), removal of implants (down arrow), and cholesterol (Chl) or testosterone (T) implantation into the third ventricle (imp) of castrated Japanese quail. Values are the means of six birds (Chl) or five birds (T) and bars indicate the standard errors of the means.

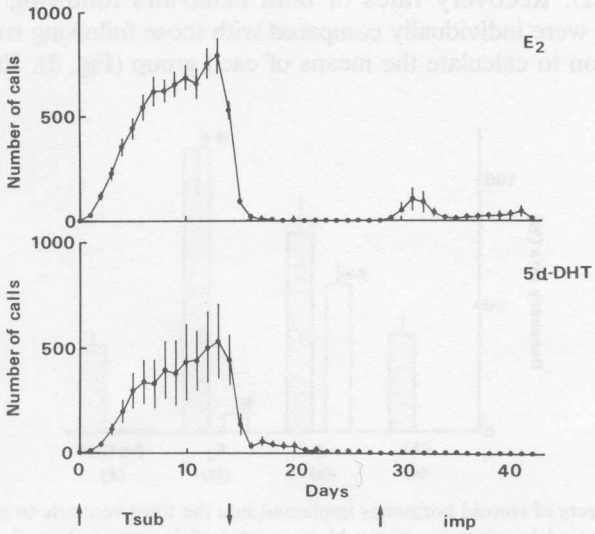

FIG. 2. Changes in numbers of calls during subcutaneous testosterone implantation (Tsub), removal of implants (down arrow), and estradiol-17β (E_2) or 5α-dihydrotestosterone (5α-DHT) implantation into the third ventricle (imp) of castrated Japanese quail. Values are the means of 11 birds (E_2) or 6 birds (5α-DHT) and bars indicate the standard errors of the means.

TABLE 1
Weights of Micropellets of Steroid and Paraffin Mixtures which are the Same Size as Those Implanted into the Third Ventricle

Steroids in pellet	No.	Weight (μg)
Cholesterol	5	497 ± 24.0[a,b]
Testosterone	5	433 ± 29.2
Estradiol-17β	5	517 ± 26.4
5α-Dihydrotestosterone	5	475 ± 8.0

[a] Mean ± SEM.
[b] Since steroids and paraffin were mixed 1:2 by weight, actual weights of hormones implanted were one-third of the mixtures.

as in implantation. Since the mixtures consist of steroids and paraffin 1:2 by weight, actual weights of hormones implanted are about 150 to 170 μg.

T pellets were effective in inducing calling; the restored level was more than half of that observed in subcutaneous implantation (Fig. 1). Profiles of development of calling after ventricular implantation were not basically different from those of subcutaneous implantation. However E_2 pellets had very slight effects, if any, in inducing calling (Fig. 2). 5α-DHT was completely ineffective in inducing calling after direct application into the brain (Fig. 2). Recovery rates of both behaviors following ventricular implantation were individually compared with those following subcutaneous T implantation to calculate the means of each group (Fig. 3). Effectiveness

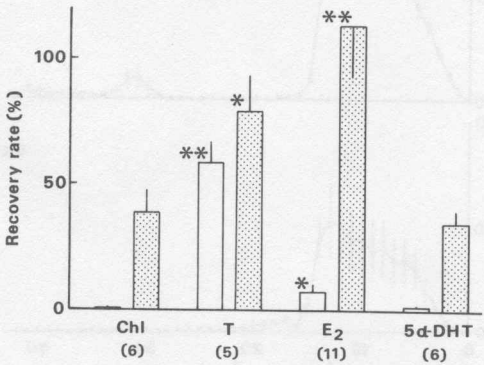

FIG. 3. Effects of steroid hormones implanted into the third ventricle to recover calling (white column) and locomotor activity (dotted column) in castrated quail. The data are expressed as percentage recovery rates during the ventricular implantation considering the values of the subcutaneous T implantation as 100%. The values are the means of the number indicated in parenthesis under each hormone with standard errors (bars). Significant differences from the cholesterol group are shown: $*P < 0.05$ and $**P < 0.01$ (t test, one tailed). Abbreviations are the same as in Figs. 1 and 2.

of T with ventricular implantation was 58.3% of T subcutaneous implantation. E_2 was about 7% and 5α-DHT was 1% (Fig. 3).

Since steroid implantation into the third ventricle resulted in much individual variation in locomotor activity, overall profiles of changes in locomotor activity during the experimental period are not shown here; only comparisons with the subcutaneous T session are presented (Fig. 3). Locomotor activity was enhanced by ventricular implantation of T and E_2 (Fig. 3). Ventricular E_2 implants were more effective than ventricular T implants, and almost the same, or slightly more effective than subcutaneous T implants (Fig. 3). 5 α-DHT was again not effective in enhancing locomotor activity compared with cholesterol controls.

In ventricular T-implanted birds, rhythms of calling and locomotor activity per day were similar to those found in subcutaneous T-implanted birds (Fig. 4). They called most frequently just around the onset of light. Locomotor activity was almost confined during the light period making

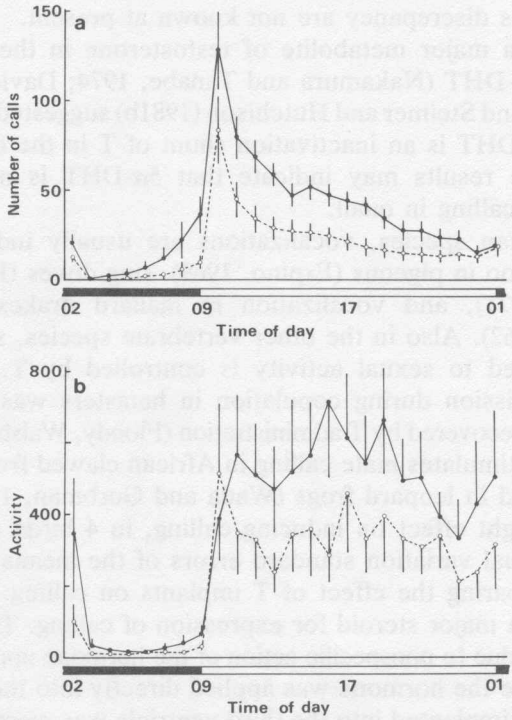

FIG. 4. Changes in numbers of calls (a) and locomotor activity (b) during a day in five castrated birds during T subcutaneous implantation (solid line) and T implantation into the third ventricle (broken line). Values were obtained by first calculating the mean for each of the five birds of the last 5 successive days of treatment, then calculating the means and SEM of these means.

a broad peak at midday. A rhythm of locomotor activity after E_2 ventricular implantation was similar to that of T implants.

DISCUSSION

These results demonstrate that steroid hormones act directly on the central nervous system to induce calling and enhancement of locomotor activity, and that different steroids influence behavior in different ways.

There is some controversy among investigators on which steroid hormones activate which behavior, especially the induction of vocalizations in Japanese quail. Adkins and Pniewski (1978) suggested that injection of 5α-DHT was more potent than T in inducing calling in quail. Deviche and Schumacher (1982) also suggested 5α-DHT was the active hormone inducing calling. However, Wada (1982) demonstrated that T but not 5α-DHT induced calling using Silastic implantation and the automated recording system as used here. In the present experiment, 5α-DHT was applied directly into the brain and found negative in inducing calling in this species, whereas T was effective (Figs. 1 and 2). However, the exact reasons for this discrepancy are not known at present.

5β-DHT is a major metabolite of testosterone in the hypothalamus rather than 5α-DHT (Nakamura and Tanabe, 1974; Davies, Massa, and James, 1980), and Steimer and Hutchison (1981b) suggested that a pathway from T to 5β-DHT is an inactivation shunt of T in the central nervous system. These results may indicate that 5α-DHT is not involved in expression of calling in quail.

In other avian species, vocalizations are usually induced by T; T induces bow-coo in pigeons (Erpino, 1969), ring doves (Erickson, 1970; Hutchison, 1971), and vocalization in mallard drakes (Phillips and McKinney, 1962). Also in the other vertebrate species, sound emission which is related to sexual activity is controlled by T. For example, ultrasound emission during copulation in hamsters was eliminated by castration and recovered by T administration (Floody, Walsh, and Flanagan, 1979). T also stimulates mate calling in African clawed frogs (Kelley and Pfaff, 1976) and in leopard frogs (Wada and Gorbman, 1977).

E_2 had a slight effect on inducing calling, in 4 birds among 11, and due to individual variation standard errors of the means are very large (Fig. 2). Comparing the effect of T implants on calling, it can be said that E_2 is not a major steroid for expression of calling. The slight effect of E_2 might be due to nonspecific action of the hormone upon the androgen receptors, since the hormone was applied directly into the brain. On the other hand, E_2 implanted into the third ventricle was more effective than subcutaneous T in enhancing locomotor activity (Fig. 3). The effect of E_2 was quite dramatic since T brain implants could recover only 80% of that by T subcutaneous implantation. In the previous experiment using subcutaneous Silastic implants, E_2 was not as effective as T (Wada,

1982). This might be explained as insufficient release of E_2 from the subcutaneous implants due to different rates of penetration of steroids through Silastic tubing. Recently, avian brain tissue has been shown to aromatize T to E_2 (Steimer and Hutchison, 1980, 1981a). Aromatase activity is high in the anterior hypothalamus and the preoptic area, very low in the areas adjacent to the hypothalamus, and negligible in the forebrain. The results support the notion that E_2 is an active metabolite of T in enhancing locomotor activity.

Where then does ventricularly implanted T or E_2 act? It is not likely that implanted hormones leak from the third ventricle and have their effects via systemic circulation, since the cloacal protrusion of T or 5α-DHT-implanted birds did not grow. Implanted steroids should act directly on the hypothalamus and its vicinity. Autoradiographic studies in avian species showed that T or E_2 was accumulated in the preoptic area, the anterior and posterior hypothalamus, the archistriatum, and in the midbrain vocal center, nucleus intercollicularis (Meyer, 1973; Zigmond, Nottebohm, and Pfaff, 1973; Barfield, Ronay, and Pfaff, 1978). Some of these areas are involved in expression of steroid-related behavior. By implanting hormones directly into these regions, the preoptic area has been shown to be essential for copulatory behavior in capons (Barfield, 1969) and courtship behavior in ring doves (Barfield, 1971; Hutchison, 1971). However, T implanted into the preoptic area or the nucleus intercollicularis did not induce crowing in capons (Phillips and Barfield, 1977). So far, it is not clear which neural substrate is sensitive to the hormone for induction of crowing in this species. As to general locomotor activity, how the enhancement is triggered is totally unknown. Since the discrete areas in the brain take up steroids, a certain site might be responsible for expression of these behaviors.

Wada (1981) showed daily rhythmic appearance of calling and locomotor activity in quail. These patterns were not related to fluctuations of circulating testosterone. In the present experiment, the birds in which T was implanted into the third ventricle also showed certain patterns of calling and locomotor activity which were the same as those observed in intact birds under a light–dark cycle (Fig. 4). This may suggest that one of the internal drives for these behaviors is a biological oscillatory mechanism and that T is only a "switch" turning from a resting state to a readily excitable state.

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