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EFFECT OF SEVERAL ANDROGENS ON TESTOSTERONE METABOLISM IN THE BRAIN AND CREST OF MALE CHICKS

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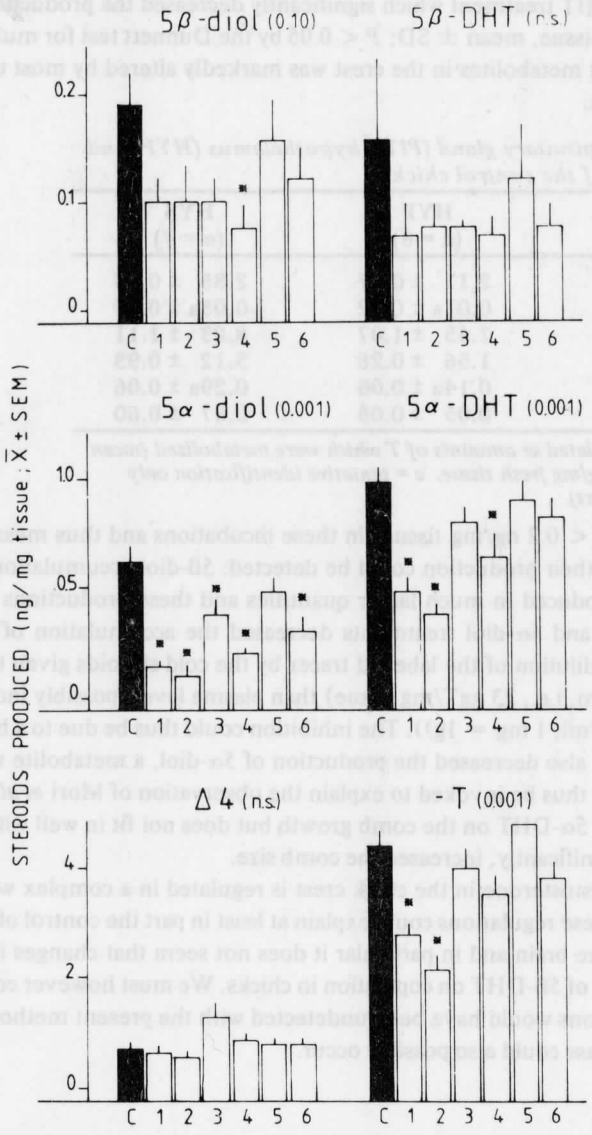
Testosterone (T) intracellular metabolism is a critical step in the action of the hormone. In mammals as well as in birds, T-induced sexual behaviour is thought to result from the action of estradiol, eventually in combination with 5α-dihydrotestosterone both derived from T metabolism (aromatization and 5α-reduction) in the brain (1). However in all avian species studied so far, brain T metabolism mainly consists in a 5ß-reduction of the steroid leading to the formation of 5ß-dihydrotestosterone (5ß-DHT) and 5ß-androstane-3α, 17ß-diol (5ß-diol) (2). It is generally believed that these compounds are devoid of any androgenic activity (no effect on sexual behaviour, nor gonadotrophin feedback, nor growth of androgen-dependent structures) so that 5ß-reduction is considered as an inactivation pathway for T (3, 4). We showed however that 5ß-DHT strongly stimulates copulatory responses measured in a hand-trust test (5) in young male chicks (6, 7) which raised the question on the mode of action of 5ß-DHT on this behavioural response. A possible mechanism could be that treatment with 5ß-DHT alters the metabolism of T in the brain and thus enhances the behavioural effects of the low subthreshold endogenous levels of T secreted by the juvenile gonads and/or adrenals (8). This hypothesis was tested in the present experiment in which the effects on T metabolism of T and five of its metabolites known to occur in the chick brain were assessed.



Testosterone metabolism in the crest of 3 week-old chicks treated with 6 different androgens and in corresponding control birds (black columns). Treatments: C = control; 1 = T; 2 = 5α-DHT; 3 = 5ß-DHT; 4 = 5α-diol; 5 = 5ß-diol; 6 = δ_4. The amounts of metabolites produced or of testosterone metabolized in the 7 groups of animals were compared by one-way analyses of variances and the P *values are reported in parentheses next to the name of the metabolites (n.s. = not significant). The experimental groups (1 to 6) were then compared to the controls by Dunnett'stest for all comparisons with the control:* $^*P < 0.05$.

Materials and methods: Male Hubard chicks were obtained on day 1 of life from a local hatchery and when 4 days old were divided into 7 groups of 5–7 birds which were implanted with 50 mm Silastic tubes (length, 2 × 25 mm; i.d., 1.02 mm; O.d., 2.16 mm; Dow Corning silastic tubing) filled with one of the following steroids: testosterone (T), 5α-dihydrotestosterone (5α-DHT), 5ß-dihydrotestosterone (5ß-DHT), 5α-androstane-3α, 17ß-diol, (5α-diol) 5ß-androstane-3α, 17ß-diol (5ß-diol), androstenedione (Δ_4) or with empty control tubes (C). The effects of these treatments on sexual behaviour, plasma LH, testes weight and crest size were assessed as described in (6). Briefly it was shown that T, 5ß-DHT and 5ß-diol stimulated the juvenile sexual behaviour, T decreased plasma LH, T and Δ_4 decreased the testes weight and finally T, 5α-DHT, 5ß-DHT, Δ_4 and 5α-diol stimulated the comb growth (6). This gives evidence that androgens implanted in silastic capsules actually reach the brain, pituitary and secondary sexual structures as confirmed by many autoradiographic and uptake studies.

When 22 days old, the chicks were sacrificed, small pieces of their crest and hyperstriatum (HYS) as well as their anterior hypophysis (PIT) and posterior hypothalamus (HYP) were immediately dissected out and incubated *in vitro* with 4-^{14}C-testosterone (Amersham, 58 mCi/mmole; about 50 000 dpm/ml, 5 mg tissue/ml) for 3 h at 41° C. The steroids produced were then extracted with diethyl ether, chromatographed on TLC silica gel plates in chloroform:acetone:n-hexane (2:1:2) and quantitatively evaluated (see 9 for methods). In addition to the non-metabolized substrate (T), five metabolites were quantified: 5α-DHT and 5α-diol, 5ß-DHT and 5ß-diol, and Δ_4. The identity of these compounds has been con-

firmed by derivatives formation and recrystallization to constant specific activity (see 10, 11, 12) with the exception of 5α-DHT and 5α-diol which were formed in too small amounts in the PIT, HYP and HYS. Values presented for these metabolites in these tissues are thus to be considered as the radioactivity isopolar to the authentic compounds, the identification being only tentative.

Results and discussion: The metabolic pattern observed in the control birds corresponds very well with the results of previous experiments (10, 11, 12). The main enzymatic activity in the crest is the 5α-reductase while the 5ß-reductase is very active in the nervous tissues and in the pituitary gland (see table).

The 3α-hydroxysteroid dehydrogenase is especially active in the PIT which results in a high 5ß-diol/5ß-DHT ratio in this tissue. The treatment of chicks with the 6 androgens (T, 5α- and 5ß-DHT, 5α- and 5ß-diol, Δ_4) did not alter the *in vitro* T metabolism in the HYP nor in the HYS (all analyses of variance (ANOVA) are not significant, $P > 0.20$). In the pituitary gland, the *in vitro* accumulation of one single metabolite, 5ß-diol tended to be altered by the *in vivo* steroid treatments ($P < 0.10$ by ANOVA). This resulted from the effect of the 5α-DHT treatment which significantly decreased the production of 5ß-diol (from 3.27 ± 1.23 in controls to 1.28 ± 0.70 ng/mg tissue, mean ± SD; $P < 0.05$ by the Dunnett test for multiple comparisons). By contrast, the *in vitro* accumulation of most metabolites in the crest was markedly altered by most treatments with steroids and these results are detailed in the figure.

Intracellular testosterone metabolism in the crest, pituitary gland (PIT), hypothalamus (HYP) and hyperstriatum (HYS) of the control chicks

	Crest ($n = 6$)	PIT ($n = 5$)	HYP ($n = 6$)	HYS ($n = 7$)
5β-diol	0.19 ± 0.07	3.27 ± 1.23	2.17 ± 0.67	2.85 ± 0.33
5α-diol	0.63 ± 0.20	–0.09a ± 0.02	0.01a ± 0.02	–0.03a ± 0.05
– T	4.44 ± 0.77	8.03 ± 1.83	7.45 ± 1.07	8.23 ± 1.11
5β-DHT	0.16 ± 0.18	1.07 ± 0.44	1.56 ± 0.26	3.12 ± 0.93
5α-DHT	1.00 ± 0.15	0.37a ± 0.13	0.14a ± 0.06	0.29a ± 0.06
Δ_4	0.80 ± 0.24	1.34 ± 0.64	0.05 ± 0.05	0.67 ± 0.60

The table gives the amounts of metabolites which accumulated or amounts of T which were metabolized (mean ± SD) during the 3 h in vitro *incubation. Results are in ng/mg fresh tissue. a = tentative identification only (see text).*

5ß-reduced androgens were produced in small amounts (< 0.2 ng/mg tissue) in these incubations and thus measured with a relatively large error. Only one significant change in their production could be detected: 5ß-diol accumulation was decreased in 5α-diol treated birds. 5α-androstanes were produced in much larger quantities and these productions were very significantly changed by the treatments. T, 5α-DHT and 5α-diol treatments decreased the accumulation of both 5α-DHT and 5α-diol. This probably does not result from a dilution of the labelled tracer by the cold steroids given to the animals (concentration of tracer much higher (± 10 000 dpm, i.e., 23 ngT/mg tissue) than plasma levels possibly induced by silastic implants (in the physiological range i.e., 1–10 ng/ml; 1 mg = 1g)). The inhibition could thus be due to a blockade of enzymes synthesis or activity. Interestingly 5ß-DHT also decreased the production of 5α-diol, a metabolite which strongly stimulates crest growth (6). This mechanism could thus be invoked to explain the observation of Mori *et al.* (12) that 5ß-DHT significantly reduces the androgenic activity of 5α-DHT on the comb growth but does not fit in well with the fact that in our experiments (6–7), 5ß-DHT slightly, but significantly, increased the comb size.

In conclusion, this study shows that the metabolism of testosterone in the chick crest is regulated in a complex way by the metabolites of T which are produced and that some of these regulations could explain at least in part the control of crest growth. In contrast, T metabolism appears more stable in the brain and in particular it does not seem that changes in this metabolism can be invoked to explain the stimulatory effect of 5ß-DHT on copulation in chicks. We must however consider that metabolic changes localized in a small group of neurons would have been undetected with the present method and that changes in other enzymatic activities such as the aromatase could also possibly occur.

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