Psychologie animale/*Animal Psychology*
(Endrocrinologie/*Endocrinology*)

Interactions des androgènes et des œstrogènes dans le contrôle de la reproduction

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Résumé — Des cailles castrées ont été injectées avec l'œstrogène synthétique, diéthylstilbœstrol (DES) ou l'androgène synthétique, méthyltriènolone (R 1881) ou avec les deux composés simultanément. Le DES et le R 1881 ont activé le comportement sexuel, inhibé la sécrétion de FSH et de LH et augmenté l'activité de l'aromatase hypothalamique. Des effets additifs du DES et du R 1881 ont été observés au niveau de l'activité de l'aromatase et de la sécrétion de FSH. Des mécanismes contrôlés par les androgènes et les œstrogènes sont donc impliqués dans le contrôle de ces réponses.

Interactions of androgens and estrogens in the control of reproduction

Abstract — *Castrated male quail were injected with the synthetic œstrogen, diethylstylboestrol (DES) or the synthetic androgen, methyltrienolone (R 1881) or both compounds simultaneously. Both R 1881 and DES activated male sexual behaviour, inhibited LH and FSH secretion and increased hypothalamic aromatase activity. Additive effects between R 1881 and DES were observed for the induction of brain aromatase and for the inhibition of FSH secretion. As a consequence, mechanisms mediated by androgen and estrogen receptors must be involved in the control of these reproductive characteristics.*

Version Française abrégée — La testostérone (T) active le comportement copulatoire et inhibe la sécrétion des gonadotropines (LH et FSH) via transformation en ses métabolites, l'œstradiol (E 2) et la 5 α-dihydrotestostérone (5 α-DHT; [2], [17]). Ceci suggère que des mécanismes androgéniques et œstrogéniques sont impliqués dans le contrôle de ces réponses. Cependant la spécificité d'action de l'E 2 et de la 5 *a*-DHT peut être mise en doute [22]. Nous avons donc étudié l'action d'agonistes synthétiques androgéniques et œstrogéniques beaucoup plus spécifiques : respectivement la méthyltriènolone (R 1881 = 17 β-hydroxy-17 α-methyl-estra-4, 9, 11-triene-3-one) et le diéthylstilbœstrol (DES). Des cailles mâles castrées (*voir* [3] pour les techniques) ont été injectées quotidiennement pendant 25 jours avec 1 mg de T, 1 mg de R 1881, 200 μg de DES, 1 mg de R 1881 + 200 μg de DES ou avec la solution contrôle (propylène glycol). Les comportements sexuels et le chant ont été quantifiés régulièrement tout au long de l'expérience. Lors du sacrifice, du sang a été prélevé pour le dosage radioimmunologique de la LH et de la FSH ([10], [11]) et le cerveau a été disséqué pour la réalisation du dosage radioenzymatique ([19], [20], [21]) de l'activité de l'aromatase dans l'aire préoptique (POA) et l'hypothalamus postérieur (PH). Le R 1881, la T et le DES ont activé le comportement copulatoire, les androgènes étant cependant moins actifs que le DES (*fig.* 1). Le chant et la croissance de la glande cloacale sont strictement liés aux androgènes et ne sont stimulés que par le R 1881 et la T. Le DES inhibe cependant la croissance de la glande induite par le R 1881 (tableau). Le R 1881 et le DES tous deux inhibent la sécrétion des hormones gonadotropes, LH et FSH. Le traitement simultané par le R 1881 et le DES se montre extremement efficace. Il stimule également l'activité de l'aromatase dans l'hypothalamus (tableau). Ces résultats montrent donc que des mécanismes nerveux stimulés par les androgènes et d'autres stimulés par les œstrogènes sont impliqués dans le contrôle des réponses comportementales et physiologiques étudiées. Cette interaction entre androgènes et œstrogènes semble représenter un trait fondamental très répandu de l'action des

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stéroïdes sur le cerveau ([6], [7], [9], [12], [16], [23]). Les mécanismes moléculaires qui sous-tendent cette interaction sont encore mal compris (*voir* [3]) mais les agonistes synthétiques à haute spécificité représentent certainement un outil important dans l'étude expérimentale de ce problème.

INTRODUCTION. — Testosterone (T) activates male sexual behaviour and inhibits gonadotropin release by acting through its metabolites, estradiol-17β (E2) and 5α-dihydrotestosterone (5α-DHT) which are produced through aromatization and 5*a*-reduction of T respectively ([2], [17]). The activity of the aromatase is regulated by both substrate ([18], [20], [21], [24]) and product [13]. However both modes of regulation have never been identified in a same species.

According to the "aromatization hypothesis" the conversion of circulating androgens to estrogens in the brain is critically involved in the activation of male sexual behaviour and in the control of gonatropins secretion (LH and FSH). It has been shown in addition that 5α-DHT enhances the behavioural effects of subthreshold doses of E2 on behaviour and has effects on its own on plasma gonadotropins, especially LH.

However, the specificity of action of E2- and 5α-DHT-treatments is doubtful. On one hand, both hormones are metabolized into other products in the central nervous system and this might modulate their action. On the other hand, the specificity of their action at the receptor level is questionable. Estradiol, for example, is an effective competitor for the binding of T to the hypothalamic androgen receptor and as a consequence might mimic androgenic effects at high concentrations [22]. One strategy to separate the action of androgens from that of estrogens is to use very specific synthetic compounds which mimic their actions. Amongst these compounds are the synthetic androgen methyltrienolone (R 1881 = 17β-hydroxy-17α-methyl-estra-4, 9, 11-triene-3-one) and the synthetic estrogen diethylstilbestrol (DES). These synthetic agonists show high specificity at the receptor level and are not readily metabolized in the brain.

DES has been shown to activate male sexual behaviour in rat [8] and quail [1] and to increase preoptic aromatase activity in doves [13]. These results suggest that an estrogen receptor mediated mechanism is involved in the activation of these characteristics. R 1881 facilitates male sexual behaviour in rats [15] and quail [3] suggesting that an androgen receptor dependent mechanism is also involved in the activation of copulation. R 1881 also inhibits plasma LH more effectively than T [3]. However, R 1881 and DES have never been given simultaneously to gonadectomized animals on order to detect synergistic effects of androgens and estrogens on reproductive characteristics. The aim of the present study was to determine the actions of DES and R 1881, when administered alone or in combination, on preoptic aromatase activity and LH and FSH levels, as well as on sexual behaviour in castrated males in order to determine the relative importance of both androgens and estrogens in the activation of these responses.

MATERIAL AND METHODS. — *Experimental procedure.* 32 castrated male quail (*Coturnix coturnix japonica; see* 3 for details on surgery and housing of the birds) were randomly assigned to 5 experimental groups which daily received one of the following injections: testosterone (T; Sigma T-1500) 1 mg ($n=7$), 17β-hydroxy-17α-methyl-estra-4, 9, 11-triene-3-one (R 1881; generously supplied by Roussel-Uclaf, Romainville, France) 1 mg ($n=7$), diethylstilbestrol (DES; Sigma D-4628) 200 μg ($n=7$), R 1881-1 mg + DES-200 μg ($n=6$) or a control solvent injection ($n=5$).

All injections were in 100 μl of propylene glycol and were performed every morning for 25 days. The amount of hormone administrated was based on previous results ([1], [3]). The day before sacrifice, birds were weighed and their cloacal gland area was measured with a caliper (greatest length × greatest width). At

sacrifice, blood samples were collected for the assay of serum LH and FSH, brains were removed for the assay of hypothalamic aromatase and the completeness of castration was checked for all birds.

Behavioural tests. — Throughout the experiment, male sexual behaviour was quantified every other day (total of 12 tests) during a 5 min. presentation to a stimulus female. Latencies and frequencies of the following behaviour patterns were recorded: neck-grab (NG), mount attempt (MA), mount (M) and cloacal contact movements (CCM; see 14 for a detailled description).

Birds were also tested for receptivity the day before and the day of sacrifice. The male to be tested was first introduced into the arena and 30 sec. later a stimulus male was asded. Receptive behaviour was recorded for 5 min. and quantified on a non-parametric scale ranging from 1 to 3 with 1 = no receptivity (running away, fighting), 2 = partial receptivity (standing still) and 3 = full receptivity (squat).

Beginning 1 week after the start of hormone treatments, birds were also tested each day for the occurence of crowing. We counted in the home cages the number of crows performed by each bird in the course of a 10 min. period. Quail were observed in a random order in groups of 8 during differents of the lights-on portion of the light-dark cycle.

LH and FSH assay. — Serum LH and FSH concentrations were measured using radioimmunoassays already described and validated ([10], [11]). The reference standards were NIAMDD-rat FSH-RP1 for FSH and chicken LH fraction AE1 (CHLH) for LH.

Dissection of brain samples and aromatase assay. — Aromatase activity was measured in the preoptic area (POA) and posterior hypothalamus (PH) of each bird by a radioenzymatic technique previously described ([19], [20], [21]) which involves incubation of the brain homogenate in the presence of tritiated testosterone and quantification of the radioactive œstradiol produced following its extraction by organic solvents and purification by phenolic partition and thin layer chromatography.

RESULTS. — No difference in body weight between groups was observed at the beginning nor at the end of the experiment (both ANOVAS were not significant).

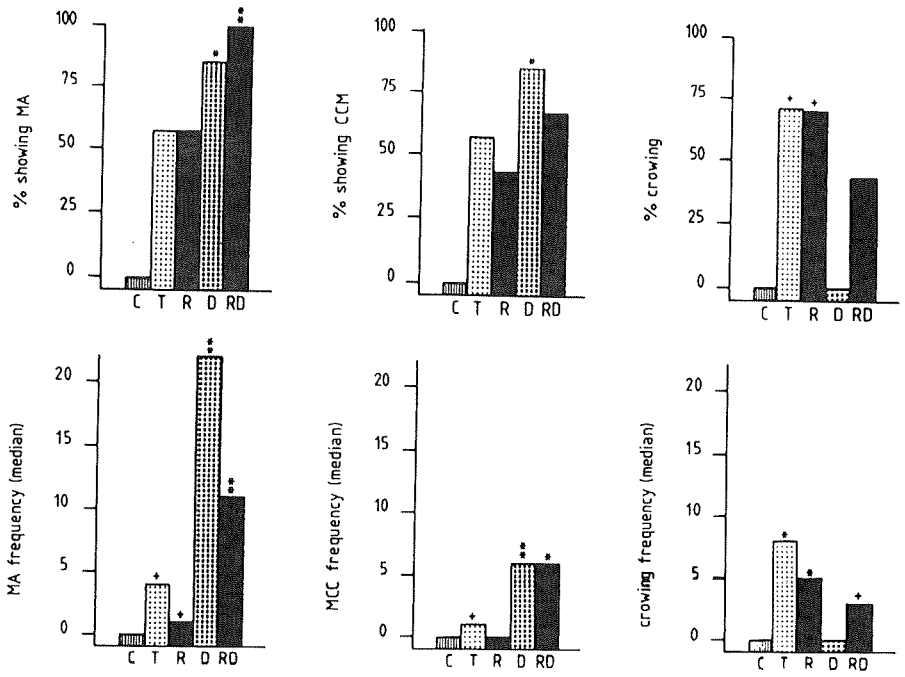
Both T and R 1881 activated mount attempts and cloacal contact movements in about 50% of males, but the differences compared with the control group was not significant (*Fig.* 1). By contrast, DES was very effective in stimulating male sexual behaviour (86% of active birds). As a consequence no additive effects between DES and R 1881 could be observed. In contrast to male sexual behaviour, crowing was significantly activated by T and R 1881, but not by DES. Only DES-treated males showed receptive behaviour (at least score 2, *see* material and methods for definitions). However, the difference with the control group was not fully significant (control birds: 0/5; DES-birds: 5/7 males were receptive, $2p < 0.053$ by two-tailed Fisher exact probability test). There were no receptive birds in the T-group and R 1881-group.

At the end of the experiment, cloacal glands were much more developed in androgen-treated birds (T, R 1881, R 1881 + DES) than in control and DES treated animals (Table).

R 1881 was much more active than T on this response. DES significantly inhibited cloacal gland growth induced by R 1881.

At sacrifice, control birds had very high LH and FSH levels (*see* Table). The amount of T administered daily (1 mg) was not sufficient to depress gonadotropin levels. By contrast, both R 1881 and DES reduced significantly FSH and especially LH concentrations. A significant additive effect between R 1881 and DES was observed for the reduction of serum FSH.

Hypothalamic aromatase activity was higher in T-treated birds than in controls (*see* Table). A clear additive effect between R 1881 and DES was also observed for the induction of this enzyme activity in the PH and even more clearly in the POA. Whereas R 1881 and DES had little effect on enzyme activity when administered separately, they induced aromatase activity very efficiently when administered together. The aromatization was similar in T and R 1881 + DES animals than in T-animals.



Percentage of castrated male quail that showed mount attempts (MA), cloacal contact movements (CCM) and crowing and median frequencies of these behaviors following treatment with testosterone (T), R 1881 (R), diethylstilboestrol (D) or R 1881 and diethylstilboestrol (RD). Untreated castrates (C) were used as control. Percentage of active birdswere compared by Fisher tests, behavior frequencies by Mann Whitney U-tests following a significant Kruskal Wallis analysis of variance. All probabilities are two tailled and refer to the comparison with the control (C) group. ★★ = $p<0.01$, ★ = $p<0.05$, + = $p<0.10$.

Pourcentage des cailles mâles castrées qui montrent les comportements d'essai de monte (MA), mouvement de contact cloacal (CCM) et chant (crowing) et fréquences médianes de ces comportements après traitement par la testostérone (T), le R 1881 (R), le diéthylstibœstrol (DES) ou le R 1881 associé au DES (RD). Des oiseaux castrés non traités (C) sont utilisés comme contrôles. Les pourcentages d'oiseaux actifs ont été comparés par le test de Fisher et les fréquences comportementales par le test U de Mann-Whitney suite à une analyse de variance (Kruskal-Wallis), significative. Toutes les probabilités sont bilatérales et concernent la comparaison avec le groupe C. ★★ = $p<0,01$, ★ = $p<0,05$, + = $p<0,10$.

Discussion. — These results are in agreement with the hypothesis that mechanisms controlled by androgen and estrogen receptors are both involved in the activation of male sexual behaviour, the inhibition of gonadotropin secretion and the induction of hypothalamic aromatase activity. As in a previous study [3], R 1881 at the dose of 1 mg/day was as efficient as T in activating copulation. DES activated high levels of male sexual behaviour. Crowing and cloacal gland development, which are strictly androgen-dependent characteristics [3], were only activated by T and R 1881. As shown previously [3], R 1881 was more efficient than T in activating cloacal gland growth which can be explained by its higher affinity for the androgen receptor in comparison with T and its resistance to metabolism ([4], [5]). Interestingly, DES decreased the effect of R 1881 on cloacal gland development. We had already reported that E2 inhibits the cloacal gland growth induced by 5α-DHT [3]. This result could have been interpreted by competition between 5*a*-DHT and E2 for the androgen receptor, with E2 acting as an antagonist. As DES, in contrast to E2, does not compete for the androgen receptor, the present observation rules out this interpretation and clearly shows that an estrogen receptor mediated mechanism inhibits androgen induced cloacal gland growth.

TABLE

Cloacal gland area, serum levels of LH and FSH and aromatase activity in the preoptic area and posterior hypothalamus in castrated male quail submitted to different steroid treatments (*see* Figure for detail).

Surface de la glande cloacale, taux sériques de LH et FSH et activité de l'aromatase dans l'aire préoptique et l'hypothalamus postérieur de cailles castrées soumises à différents traitements hormonaux (voir *la figure pour le détail*).

Variable	Groups C	T	R 1881	DES	R 1881 +DES
Cloacal gland area (mm^2)	36 +7 (^{c})	117 + 24 (^{b})	213 + 52 (^{a})	72 +27 (^{c})	142 + 40 (^{b})
LH (ng CHLH/ml)	21.3 +14.7 (^{a})	17.7 + 8.4 (^{a})	2.7 +1.8 (^{b})	6.5 +3.8 (^{b})	0.8 +0.5 (^{b})
FSH (ng FSH-RP1/ml)	475 + 55 (^{a})	441 + 27 (^{a})	281 + 90 (^{b})	317 + 93 (^{b})	164 + 13 (^{c})
Aromatase POA (fmol/mg protein)	30 + 9 (^{c})	83 +23 (^{b})	53 +27 (^{b}), (^{c})	70 +31 (^{b}), (^{c})	145 + 46 (^{a})
Aromatase PH fmol/mg protein)	31 +15 (^{c})	65 +21 (^{a}), (^{b})	41 +10 (^{b}), (^{c})	53 +23 (^{b}), (^{c})	79 +18 (^{a})

Data presented are means and standard deviations. The different groups were compared by Newman-Keuls tests following a significant analysis of variance. Means from the same line with a different superscript are significantly different at the 5% level.

Les données présentées sont la moyenne et la déviation standard. Les différents groupes ont été comparés par le test de Newman-Keuls suite à une analyse de variance significative. Les moyennes d'une même ligne qui possèdent au moins un indice différent sont significativement différentes au niveau de 5 %.

Both R 1881 and DES also inhibited LH and FSH secretion and increased the hypothalamic aromatase activity which again shows that both androgen and estrogen receptors regulate these physiological responses. Additive effects of R 1881 and DES were also observed here.

This study thus shows that both androgen- and estrogen-dependent mechanisms are involved in the control by the brain of reproductive characteristics such as male sexual behaviour, gonadotropin release and hypothalamic aromatase activity. Previous studies have demonstrated the same hormonal requirements for other brain functions such as the vasopressinergic innervation of the adult rat brain [7] and numerous behaviours including olfactory recognition in the male hamster [23], aggression in guinea pigs [12] and male mice [9] and singing in canaries [6]. Both androgens and estrogens are also involved in the process of sexual differentiation [16]. This raises the important question of the mechanisms by which both androgens and estrogens exert facilitatory central effects on a wide array of physiological responses. The two types of steroids might act on the same or on different brain cells located in the target nuclei. They could even exert their effects by modifying the activity of neurons which could be quite distant from the target zone. The molecular mechanisms by which androgens and estrogens produce their combined effects on the brain are also unclear. Numerous brain functions, probably controlled by different parts of the brain require the simultaneous activation by

androgens and estrogens thus suggesting that this is a basic principle of brain physiology. Biochemical studies suggest several molecular mechanisms by which androgens and estrogens might exert their combined effects. In the presence of E2, the metabolism of androgens by the brain is modified and this could affect their behavioural action (*see* [3] for detail). Androgens and estrogens might also exert synergistic actions at the level of gene transcription. The mechanisms by which both androgens and estrogens facilitate several brain functions have now to be discovered. The use of highly specific and synthetic hormones represents an important tool as it allows to separate the actions of estrogens from that of androgens. The observation that numerous brain characteristics are under the control of both types of hormones suggests the presence of a common and fundamental mechanism.

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