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Effects of Apomorphine on Sexual Behavior in Male Quail

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ABSIL, P., S. DAS AND J. BALTHAZART. *Effects of apomorphine on sexual behavior in male quail.* PHARMACOL BIOCHEM BEHAV 47(1) 77–88, 1994.—In the rat, dopamine (DA) facilitates male copulatory behavior. Indirect evidence based largely on neuroanatomical data suggest that in quail DA is also implicated in the control of male reproductive behavior but there is no pharmacological evidence to support this conclusion. To test this idea, castrated testosterone (T)-treated male quail were injected with various doses of the dopaminergic agonist apomorphine (APO) in the range 1–10,000 μg/kg. The sexual behavior of birds was recorded starting 15 min after APO injection for a duration of 30 min. A dose-dependent inhibition of male reproductive behavior that lasted for the entire duration of the test was observed. In a second experiment, gonadectomized T-treated male Japanese quail were injected daily with APO (0, 10, or 1,000 μg/kg) during 8 days. Their sexual interactions with a partner were quantified either 24 h or 15 min after the last injection. No influence of the treatment on copulatory behavior was observed 24 h after the last injection, but a strong inhibition was present when the test was performed 15 min after. To research whether the inhibitory effects of APO were due to a preferential action on D_2 presynaptic autoreceptors, male quail were pretreated with two different D_2 antagonists (spiperone or pimozide; 0.5 or 2 mg/kg) before being injected with APO (100 μg or 1 mg/kg). Spiperone facilitated male sexual behavior but did not suppress the inhibitory effect of APO. No significant effect of pimozide was observed. These results support the notion that DA modulates male sexual activity in the Japanese quail. The specific role of the different dopaminergic receptor subtypes remains, however, to be elucidated.

Male sexual behavior Dopamine Catecholamines Apomorphine Japanese quail D_2 antagonist Preoptic area

NUMEROUS studies performed mainly in the rat have shown that dopamine (DA) is implicated in the control of male sexual behavior. These experiments suggest that an increased dopaminergic transmission in the brain facilitates copulation in males. This behavior is indeed enhanced by systemic as well as central injections of dopaminergic agonists such as *l*-dopamine (l-DOPA) or apomorphine (APO) (21,22). This conclusion is supported by studies using dopaminergic antagonists that were shown to disrupt male copulatory behavior (21,22). Electrolytic or chemical lesions of the central dopaminergic system also adversely affected copulation in the rat (22). Clinical data also suggest that DA could facilitate reproductive behavior in the human male [for review, see (21)]. Further evidence linking DA and male sexual activity comes from measures of the brain content of DA and its metabolites (22). Recently, in vivo voltammetry and in vivo microdialysis have been used to monitor DA synthesis and release during sexual interactions. A marked increase in DA levels was observed in response to the presence of the sexual partner and during copulation itself (22).

The neuroanatomical site of DA action on the different aspects of male copulatory behavior has been partly identified. For a long time, the medial preoptic area (MPOA) and limbic regions such as the nucleus accumbens have been known as important sites mediating the expression of reproductive behavior in the rat (31,37,43). Stereotaxic infusion of dopaminergic drugs into these brain areas suggests that DA terminals in the MPOA could facilitate consummatory aspects of the behavior reflected in measures of the copulatory rate, ejaculation, and penile reflexes (22,38–40,54,67). The innervation of the MPOA mediating these effects would originate from the zona incerta and would be part of the incertohypothalamic tract (29,42). By contrast, anticipatory aspects of male sexual behavior would depend on the dopaminergic transmission in the nucleus accumbens (22). This and other limbic structures receive projections from the ventral tegmental area through the mesocorticolimbic system (29,42).

Based on their pharmacological properties, two major types of DA receptors, D_1 and D_2, have been identified in the rat brain (27). Both types appear to be implicated in the con-

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trol of male sexual behavior (39,40). More recent studies using essentially molecular biology techniques have distinguished five receptor subtypes labeled D_1 to D_5 (29,42,60,61). Their pharmacological characterization is still in progress. It is currently admitted that the activation of postsynaptic dopaminergic receptors (mainly D_1) enhances male sex behavior while the stimulation of presynaptic autoreceptors (mainly D_2) has the opposite effect. Most DA agonists and antagonists are not totally selective and may act on both types of DA receptors to alter the behavioral output. Therefore, the effect of a specific drug depends on its relative pre- and postsynaptic actions. This mechanism is often invoked to explain the differential effects of various doses of dopaminergic drugs on male reproductive activity (21). For example, low doses of APO would bind preferentially to the presynaptic autoreceptors that are more sensitive to dopaminergic agonists (36,44,46). This could explain why they inhibit sexual activity. By contrast, higher doses of this agonist would be able to stimulate postsynaptic receptors and, therefore, augment dopaminergic transmission, which would enhance male reproductive behavior.

Little work has been devoted to the relation between DA and avian behavior (47). One study demonstrated that aggressive behavior is positively correlated with DA levels in the brain of the Japanese quail (32). In pigeons, the dopaminergic agonist APO seems to be able to induce pecking while in the chicken IM injections of APO increase locomotor activity, pecking, and vocalization [for review, see (47)]. However, no pharmacological study has been carried out to specifically investigate the possible implication of DA in the regulation of reproductive behavior in birds. Several studies in the Japanese quail suggest, nevertheless, that this neurotransmitter could play a key role at this level.

In quail, the DA turnover in the preoptic area (POA) (49) and more specifically in the sexually dimorphic medial preoptic nucleus (POM) is sexually differentiated (15). The depletion of DA after an injection of α-methyl-para-tyrosine (αMPT) is higher in males compared to females. The higher DA turnover in the POM is thought to be indicative of a higher dopaminergic activity. This sex difference is observed in sexually mature birds but also in birds gonadectomized and submitted to a replacement therapy with testosterone (T). The sex difference is therefore present when adult males and females are placed in the same endocrine conditions (e.g., gonadectomized birds). The sex difference of DA turnover seems therefore to be organizational in nature: It would depend on the early action of gonadal steroids rather than on their influence in adulthood.

This neurochemical sex difference is potentially relevant to the control of male copulatory behavior. The POM is indeed a necessary and sufficient site of T action in the activation of this behavior in adult males (19). Females, by contrast, never show a male-type sexual behavior in response to T and this behavioral dimorphism results from the early action of estrogen in females: They are demasculinized by their endogenous ovarian steroids (2,4,12,56,58). Because DA stimulates male sexual behavior in rats (21,22), DA turnover in quail is significantly higher in the male than in the female POM (15), and this dimorphism is insensitive to the adult hormonal status (15), it can be speculated that the turnover difference plays a causal role in the control of the behavioral difference in quail.

In the brain, T must be aromatized into estrogen to activate male sexual behavior and this enzymatic process is a limiting factor in the action of the steroid (13,14,17,20). This behaviorally relevant aromatization of T takes place in the POM (19,20). It is therefore interesting to note that the aromatase activity (AA) is also sexually differentiated in the quail POA: It is higher in males than in females and this enzymatic difference is still present in gonadectomized birds submitted to a same hormonal replacement therapy with T (14,57). It is assumed to be also organizational in nature. The exact control mechanisms for the enzymatic difference are, however, unknown at present.

Dopaminergic receptors of D_1 and D_2 subtypes have been observed in the quail brain by autoradiography using [^{3}H]-SCH23390 and [^{3}H]raclopride as ligands. Highest levels of binding were found in the basal ganglia. Areas implicated in the control of reproduction, such as the POA and hypothalamus, were labeled to a lesser extent (1). The D_1 receptor subtype is linked to the production of cyclic adenosine monophosphate (cAMP) by a stimulatory guanosine binding protein (G protein) (42). The second messenger cAMP can modulate AA in several tissues including the brain (25,26,45,66). Thus, it is tempting to think that DA could modulate AA in the brain by binding to D_1 receptors and that the sex dimorphism in AA that is present in the quail POA could be a consequence of the sex dimorphism in dopaminergic activity in this area. In support of this notion, tyrosine hydroxylase-immunoreactive fibers were recently observed in close contact with aromatase-immunoreactive cells in the POM and in the region of the nucleus accumbens-nucleus striate terminalis (9). This neuroanatomical association could represent the substrate for the regulation by catecholamines of T aromatization, a limiting step in the behavior activation by T.

However, as mentioned above, no pharmacological manipulation of the dopaminergic system in quail has been performed so far to establish the potential role of this transmitter in the control of male sexual behavior. We present here a series of three experiments that were carried out to test the idea that DA is implicated in the control of male reproductive behavior, and research whether dopaminergic neurotransmission has, like in mammals, a facilitatory role at this level.

METHOD

Experiment 1: Injection of Apomorphine

Animals. This experiment was carried out on 10 male Japanese quail (*Coturnix japonica*) that were bought from a local dealer (Dujardin, Liernu) at the age of 3 weeks. Birds were castrated under total anesthesia (Hypnodil, Janssen Pharmaceutica, Beerse, Belgium, 15 mg/kg) approximately 1 week later. The two testes were removed through a unilateral incision behind the last rib. Quail were then left undisturbed in individual cages for about 3 months, when the actual experiment was begun. Throughout their life at the laboratory, birds were exposed to a simulating photoperiod (16 L : 8 D). They always had food and water available ad lib.

Drugs. The dopaminergic agonist apomorphine HCl (APO; Sigma A-4393, Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water containing 0.2% ascorbic acid at doses ranging from 1 μg to 10 mg/ml and injected (1 ml/kg) IP (just in front of the leg, in the inguinal fold) to animals. Doses in the range of 1 μg to 10 mg/kg body weight (1, 10, 100, 1,000, and 10,000 μg) were tested for their behavioral effects. On any given experimental day, half the birds were injected with APO while the other half received the vehicle. On the next day, this distribution was reversed so that after two consecutive tests (one round) each of the 10 birds had been treated once with APO and once with the vehicle. In this way, each bird served as its own control (C condition). For

each dose of the dopamine agonist except the 10-mg/kg dose, two series of two tests (two rounds) were conducted in this way. Birds were first tested during two rounds with the dose of 100 μg/kg in the absence of steroid treatment. Because no sexual behavior was observed, it was decided to give birds a pretreatment with a low dose of T to optimize the condition for the demonstration of a stimulatory effect of APO. Each male was therefore implanted SC with a 5-mm long Silastic capsule (Silastic 602-265, Medical-Grade Tubing, Dow Corning) filled with crystalline T (Sigma T-1500) and closed with Silastic glue (Silastic Brand 891, Silicone type A, Dow Corning). This implant was left in place for the entire duration of the experiment and behavioral testing took place between 4 and 46 days after the implantation (see below for detail). It has been shown previously that this represents the minimal dose of T that will reinstate a weak copulatory behavior in castrated male quail (14,18).

The different doses of APO were tested in the following order: 100 μg (4–10 days after T implantation); 10 μg (12–18 days after T implantation); 1 μg (20–27 days after T implantation); 1 mg (30–36 days after T implantation); and 10 mg/kg (43–46 days after T implantation). The dose of 10 mg/kg was only tested during one round because side effects of the drug (e.g., tremors) were observed so that specific effects on sexual behavior could no longer be expected. Each test was separated from the following one by about 2–4 days. The order of testing for the different doses was dictated by the available data on rats. A dose in the middle of the active range was first selected. From there, lower amounts were progressively tested, and because they still did not provide the same results as in rats higher amounts of APO were used (see the Discussion section for more detail). The effects of T itself progressively increased during the entire experiment as evidenced by the behavioral data collected in the control condition. The magnitude of this variation was, however, limited so that, at each point in time a stimulatory as well as an inhibitory effect of APO could still potentially be demonstrated.

The body weight and the cloacal gland area (CGA = largest width × largest length measured with a caliper) of birds were regularly measured throughout the experiment. The cloacal gland is an androgen-sensitive structure (52) that provides an independent and easy measure of T action in quail.

Behavior testing. Fifteen minutes after APO or vehicle injection, the experimental bird was introduced into a test arena (60 × 40 × 50 cm) that contained a sexually mature female. Its sexual behavior was then observed uninterruptedly for the next 30 min. The frequencies of four sexual behaviors [neck grab, mount attempt (MA), mount, and cloacal contact movement (CCM)] were recorded for each of the six 5-min periods [see (5,41) for description].

Experiment 2: Repeated Injections of Apomorphine

Animals. Nineteen male Japanese quail were bought from the same local dealer (Dujardin, Liernu) at the age of 2.5 weeks. They were castrated as described before at the age of 3 weeks and implanted 2 weeks later with one 2.5-mm long Silastic capsule filled with T. It has been shown previously that this hormonal treatment provides a level of T at the threshold for the activation of sexual behavior in castrated male quail (14,18). The quail were then placed in individual cages. The APO treatments were started 1 month later, when the small T implants had established a stable baseline of copulatory behavior.

During their stay in the laboratory, birds were submitted to a stimulating daily photoperiod (16 L : 8 D). Food and water were provided ad lib. The body weight of subjects was regularly measured throughout the experiment. The cloacal gland of the quail was measured at the end of the treatments. Birds were then killed to check for the presence of the implant and possible regeneration of the testes.

Drugs. Birds were distributed at random into three groups and injected IP with either vehicle (distilled water containing 0.2% ascorbic acid; control group) or APO at a low or a high dose (10 μg/kg or 1 mg/kg). Each bird was injected once every day during 8 days. A control behavioral test was performed on day 1 before the first injection. Quail were then tested on days 4 and 7, 24 h after the last injection, and on day 8, 15 min after the last injection.

Behavior testing. During each test (days 1, 4, 7, and 8), the male sexual behavior of experimental subjects was recorded during 5 min as described above. It was shown in the first experiment that this duration was sufficient to record almost all the behavioral interactions of a male in presence of a sexually attractive female. This, in fact, confirmed previous studies carried out in our and in other laboratories (5,41).

Experiment 3: Injection of D_2 Dopaminergic Receptor Antagonists

Animals. Fifty-four male Japanese quail bought from our local dealer were castrated at the age of 3 weeks. At 5 weeks of age, they were implanted with a 5-mm long Silastic implant filled with T and housed in separate cages. During all experimental procedures, birds received a stimulating photoperiod (16 L : 8 D) and food and water ad lib. The weight of birds was regularly controlled to adjust the dose of pharmacological substance injected and detect a possible alteration of subjects' health.

Drugs. APO dissolved in distilled water containing 0.2% ascorbic acid (400 μg or 4 mg/ml) was injected at doses of 100 μg/kg or 1 mg/kg. Two D_2 antagonists were tested in the present experiment. Spiperone (8-[3-(*p*-fluorobenzoyl) propyl]-1-phenyl-1,3,8-triazaspirol [4,5] decan-4-one or spiroperidol; Sigma S-7395) and pimozide (1-[1-(4,4-*bis* [4-fluorophenyl]-butyl)-4-piperidinyl]-1,3-dihydro-2*H*-benzimidazol-2-one; Sigma P-1793) were first dissolved in a small amount of glacial acetic acid. Saline solution (9 g NaCl/l distilled water) was then added to obtain a final dilution of 2 mg/ml. Spiperone was injected at 0.5 mg/kg or 2 mg/kg. All birds that were not injected with a given drug always received an injection of the corresponding control vehicle solution (2 mg ascorbic acid per ml of distilled water or 20% acetic acid in saline; 250 μl/kg). All solutions were freshly prepared and kept on ice before the injection. Quail were distributed into six experimental groups of nine birds each. On each experimental day, all animals received two IP injections: They were first injected with one D_2 antagonist or its vehicle (V1) and then, 15 min later, they were injected with APO or its vehicle (V2). Six different experimental treatments were defined in this way: control (C group; V1 + V2); apomorphine alone (APO group; V1 + APO); pimozide alone (PIMO group; PIMO + V2); spiperone alone (SPIP group, SPIP + V2); APO + pimozide (APO + PIMO group); and APO + spiperone (APO + SPIP group). Three series of behavioral observations were carried out after the injection of three separate combinations of APO and D_2 antagonists: low dose of APO (100μg/kg) associated with low (0.5 mg/kg) or high (2 mg/kg) doses of D_2 antagonists (low-D_2 and high-D_2 conditions, respectively) and high dose of APO (1 mg/kg) associated with high doses (2 mg/kg) of D_2 antagonists (high APO condition).

The body weight and cloacal gland area of birds were measured at the start and end of the experiment to provide independent estimates of the general condition of birds. Although no visible side effect of the drug could be detected, eight birds died in the course of the present experiment, higher than what is normally observed during a similar period (about 1 month) in untreated animals. Indeed, no lethality was observed in the control group while one to three birds died in almost every group treated with drugs (two in the APO group, three in the PIMO group, two in the APO + PIMO group, and 1 in the APO + SPIP group). These effects do not, however, reach statistical significance so it is impossible to establish a causal relationship between the death of subjects and the treatment to which they were submitted.

Behavior testing. Birds were tested 15 min after the second injection (APO or corresponding vehicle). Based on the results of Experiment 1, the different sexual behaviors were recorded during 5 min as described above. A preliminary control test was performed 10 days after the implantation of the Silastic capsules filled with T. The quail were then tested in the three pharmacological conditions described above (three rounds). For each round, two behavioral tests were done 3–5 days apart. Animals were left untreated for at least 2 days between the different rounds.

Data Analysis

In all experiments, the frequencies of MA and CCM and the percentage of birds showing these behavior were statistically analyzed. Because the analysis of MA and CCM always led to the same conclusions, results relative to MA only will be presented here in detail.

In the first experiment, the mean behavior frequency recorded during the two tests of each round (one round corresponding to one dose of APO; one test only at the 10-mg dose) was analyzed by a two-way analysis of variance (ANOVA) with the treatment (apomorphine/vehicle) and dose as factors. Because the same birds were used throughout, a design with two matched factors was used. The experimental and control results for each dose were subsequently compared by posthoc Student's t-test for matched samples using the relevant mean square as the basis for the comparison. The evolution in time of the behavioral frequencies during the tests was analyzed by the same procedure. The percentages of active birds were compared by the Fisher exact probability test.

The data collected on each day during the second experiment were analyzed by one- or two-way ANOVA followed when appropriate by the Fisher protected least significant difference test (Fisher PLSD). Separate analyses excluding birds that remained sexually inactive during the entire experiment were also carried out.

During the third experiment, the behavior frequencies recorded during the two behavioral tests of each round were averaged and analyzed by one- or two-way ANOVAs comparing the different pharmacological treatments. Pairs of treatments were subsequently compared by the Fisher PLSD test. For all experiments, differences were considered significant for a bilateral probability smaller than 5%.

RESULTS

Experiment 1: Injection of Apomorphine

The increasing doses of APO inhibited the T-induced male sexual behavior in a dose-dependent fashion. The mean frequencies of mount attempt observed during the tests are presented in the top panel of Fig. 1.

The general ANOVA of these data (frequency of all birds including inactive ones) revealed a significant effect of the treatment, $F(1, 9) = 20.65, p = 0.0014$, of the dose of APO, $F(4, 36) = 3.15, p = 0.0255$, and of the interaction between these factors, $F(4, 36) = 10.30, p = 0.0001$. Posthoc t-tests for multiple comparisons confirmed the presence of significant effects at the two higher doses (see Fig. 1 for detail). The 100-μg dose already caused more than a 50% decrease in the MA frequency but this difference did not reach significance because a low activity only was observed in controls during this round, which was performed first in the sequence (behavioral effects of T had not yet fully developed; see the Method section). A separate analysis of these data by a t-test for matched samples also suggested an effect of the drug at the 100-μg dose ($t = 2.90, p = 0.0176$). Because the highest dose of APO had induced nonspecific effects (motor disturbances, feather ruffling), a separate ANOVA was run again after the exclusion of the corresponding data. This analysis confirmed the significant effect of the treatment, $F(1, 9) = 17.48$, $p = 0.0024$, the dose, $F(3, 27) = 2.94, p = 0.0512$, and their interaction, $F(3, 27) = 9.56, p = 0.0002$.

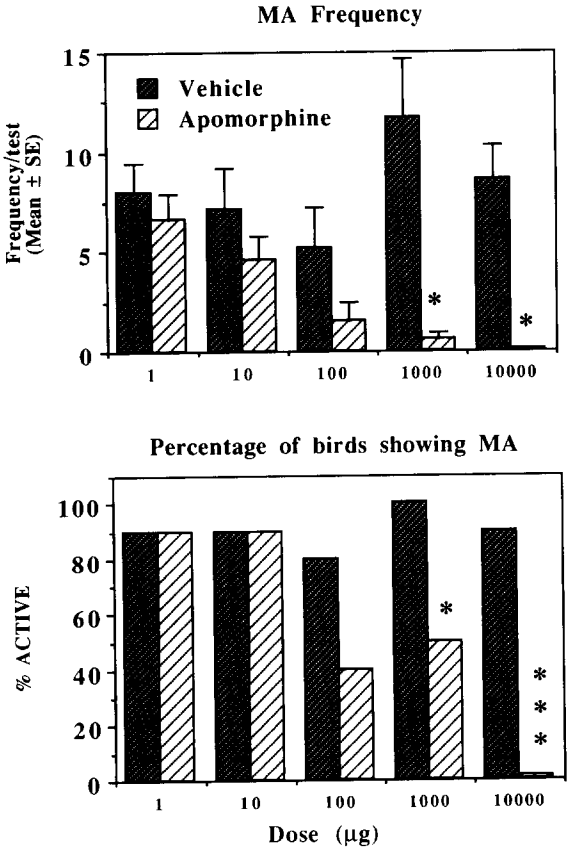

FIG. 1. Mean frequencies (top) and percentage of quail (bottom) showing mount attempts (MAs) after treatment with various doses of apomorphine (APO) or injection of the control vehicle solution. Frequencies were analyzed by two-way analysis of variance (ANOVA) followed by posthoc t-test for matched data (see text). Numbers of active birds in the APO and control conditions were compared by the Fisher exact probability test. Results of these tests are indicated at the top of the corresponding bars (*$p < 0.05$; ***$p < 0.001$).

The percentage of birds displaying MA during behavioral tests was similarly inhibited by the APO treatment (see Fig. 1, bottom panel). This effect reached statistical significance for the two higher doses ($p \leq 0.05$ by the Fisher exact probability test). The decrease in MA frequency that was observed in the entire group following injection of APO (Fig. 1, top panel) did not only reflect the decrease in the number of active birds (Fig. 1, bottom panel). APO also decreased the MA frequency in birds that remained active (data not shown). This effect was significant at the 1-mg dose (C 11.7 ± 9.37 vs. APO 1.2 ± 1.04, mean ± SD; $t = 2.45, p = 0.0292$).

Continuous monitoring of the body weight and cloacal gland area of birds revealed no sign of toxicity of the treatments. As normally observed in birds at that age, the mean body weight slightly increased during the experiment (from 249.3 ± 22.6 g at the start to 258.9 ± 32.7 g at the end; means ± SD) and their cloacal gland area enlarged as a result of the prolonged exposure to the T implant (from less than 50 mm^3 before the implantation of T to 236.6 ± 81.8 mm^3 at the end of the APO injections).

The behavioral data were further analyzed by subdividing each behavior test into six consecutive 5-min periods and plotting the behavioral frequencies observed in each interval. Like previously, the means observed during the two tests performed when quail were exposed to a same dose of APO were calculated for birds in the control and drug-treated conditions. This analysis was performed only on the data relative to the doses in the interval 10–1,000 μg/kg. At the lowest dose (1 μg/kg), no inhibition was observed. The inhibition observed at the highest dose (10 mg/kg) was associated with nonspecific motor disturbances so that no conclusion relevant to sexual behavior could be expected there. The data obtained in this way are presented in Fig. 2. They were analyzed by two-way ANOVA with matched factors (two factors within, zero factor between design).

In the control condition, the highest levels of behavioral activity were always observed during the first 5 min of the test. Thereafter, the MA frequency decreased and remained low until the end of the observation period. During the tests started 15 min after an injection of APO, a major behavioral inhibition was observed. It was already present at the start of the observation period and lasted for its entire duration (30 min). These qualitative conclusions were confirmed by the statistical analysis. At each of the three doses, the two-way ANOVA revealed a significant effect of the treatment [10 μg, $F(1, 9) = 6.44, p = 0.0318$; 100 μg, $F(1, 9) = 8.41, p = 0.0176$; 1 mg, $F(1, 9) = 16.74, p = 0.0027$] and time [10 μg, $F(5, 45) = 10.42, p = 0.0001$; 100 μg, $F(5, 45) = 2.71, p = 0.0319$; 1 mg, $F(5, 45) = 8.43, p = 0.0001$]. A significant interaction of these two factors was also detected for two of the APO doses [10 μg, $F(5, 45) = 3.21, p = 0.0145$; 100 μg, $F(5, 45) = 1.18, p = 0.3357$; 1 mg, $F(5, 45) = 6.07, p = 0.0002$]. The posthoc t-test for matched samples (see above) was then used to compare the behavior of birds at each time interval in the control and APO conditions. This confirmed the presence of a significant behavioral inhibition at the beginning of the tests. The statistical significance of this difference sometimes disappeared as time passed because the behavior in the control condition progressively decreased (see detail of statistics in Fig. 2). Based on these results, it was decided to limit the behavior tests to a duration of 5 min in the subsequent experiments.

A substantial variation of the behavior frequency in the control condition had been observed here from one test to the other. This effect was statistically confirmed by a one-way ANOVA with repeated measures performed on the data of the control group only, $F(4, 36) = 3.02, p = 0.0301$, and the posthoc Fisher PLSD tests indicated that the behavioral frequencies were significantly higher in control birds corresponding to the 1-mg dose than in those matched to the 10- or 100-μg dose. This effect could be interpreted in two ways. It could be due either to the variable duration of the exposure to T (the 1-mg APO tests were performed several weeks after the 10- and 100-μg tests; see the Method section) or to an aftereffect of the APO injections (the same birds were used alternatively in the control and APO conditions). The next experiment was carried out to discriminate between these possibilities.

Experiment 2: Repeated Injections of Apomorphine

The dose of T (2.5-mm long implants) that had been selected to provide a threshold activation of male copulatory

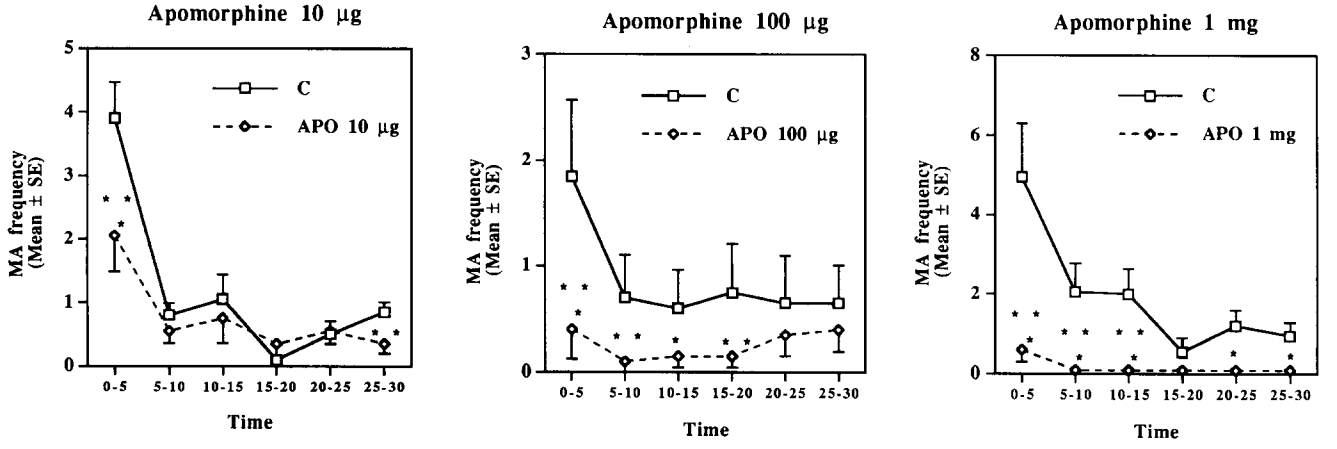

FIG. 2. Evolution in time of the mount attempt (MA) frequency in quail treated with either apomorphine (APO) at various doses (10 μg, 100 μg, or 1 mg/kg) or the control vehicle solution (C). Frequencies were analyzed by two-way analysis of variance (ANOVA) followed by posthoc t-test for matched data (see text) to compare data in the APO and control condition at various points in time. Results of these tests are indicated on the curves (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

behavior activated some mount attempts in about two thirds of the birds (12 of 19 during the pretest performed after 1 month). This percentage of active birds did not increase during the experiment due presumably to the low stimulation by T. Based on the results of this behavioral test, three matched groups of birds were constituted and repeatedly injected with either 10 μg or 1 mg APO or with the vehicle. Four active birds were included in each group so that the preexperimental behavior frequencies (MA) were statistically similar in the three groups, $F(2, 16) = 0.69, p = 0.5172$. Mean frequencies were, however, ranging form 1.83 ± 2.04 to 3.83 ± 4.12 (mean ± SD) and it was, therefore, decided to transform all behavior frequencies in percent of the mean preexperimental score of each group before any analysis. The corresponding data obtained in this way are summarized in Fig. 3.

Separate one-way ANOVAs analyzing the results of each series of tests (pretest, days 4, 7, and 8) failed to detect significant effects of the repeated APO injections, $F(2, 16) \leq 2.04$, $p \geq 0.1622$. However, similar analyses carried out only on the data relative to active birds ($n = 4$ per group) identified a significant effect of the treatments during the tests performed on day 8, that is, 15 min after the APO injection, $F(2, 9) = 4.26, p = 0.0498$. The MA frequency was significantly lower in the 1-mg than in the 10-μg APO group but the difference with the control group fell short of significance ($p < 0.10$ by the Fisher PLSD test). This test nevertheless confirmed the effect established during Experiment 1. The four active birds that had regularly shown MA during the previous observation period (day 7) remained completely inactive when tested 15 min after injection of APO at the dose of 1 mg/kg. This difference by comparison with control birds is statistically significant by t-tests including, $t(10) = 2.32, p = 0.0429$, or not, $t(6) = 3.23, p = 0.0179$, birds that were inactive during the pretest.

The experimental treatments did not affect the body weight of the three groups of birds, $F(2, 16) \leq 1,6, p \geq 0.24$, in each series of measures that were taken at different points in time. Birds in the three groups also developed small cloacal glands of similar size in response to the treatment with low doses of T, $F(2, 16) = 0.019, p = 0.9809$.

In conclusion, this experiment confirmed the short-term inhibitory effect of APO on male sexual behavior but provided no evidence for long-term effects of this agonist. The behavioral changes that had been observed in the control condition during Experiment 1 were therefore probably related to the variable duration of the exposure to T. It is unlikely that a sensitization/desensitization of dopaminergic mechanisms related to the sequential treatments with APO was implicated in this variation of the behavior.

Experiment 3: Effects of D_2 Antagonists on APO Inhibition of Male Copulatory Behavior

Experiments 1 and 2 consistently demonstrated a short-term inhibition of male sexual behavior following injection of the dopaminergic agonist APO. As discussed in the introductory section, it is often assumed that this type of behavioral effect primarily reflects an agonist action on the presynaptic autoreceptors. This idea was tested in a third experiment during which the behavioral action of APO was evaluated in birds that had been pretreated with dopaminergic D_2 antagonists. It was hypothesized that this pretreatment should reduce or abolish the potential presynaptic effects of APO.

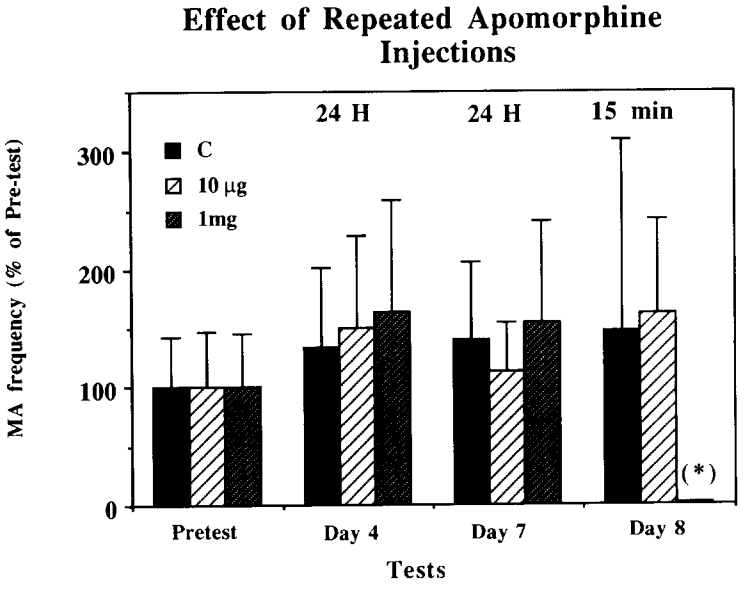

FIG. 3. Frequency of mount attempt (MA) observed in quail injected repeatedly with various doses of apomorphine (10 μg, 1 mg) or its control solution (C). Before any analysis, data were transformed in percentage of the frequencies observed during the pretest to compensate for small preexisting differences between groups. Means and SEMs are shown. Data collected on each day were analyzed by one-way analysis of variance (ANOVA) followed by Fisher PLSD tests to compare groups two by two. The results of these statistical analyses are shown at the top of the bars (*$p < 0.05$ for the specific comparison with the C group; see text).

During the pretest, which was carried out 10 days after the implantation of the T Silastic capsules, sexual behavior (at least one MA) was observed in about 60% of subjects (32 of 54). This proportion increased continuously during the experiment in parallel with the duration of the treatment with T to reach about 90% at the end. Based on the behavioral results of the pretest, birds were distributed into six matched groups and then treated with three combinations of APO and/or D_2 antagonists as described above. The mean MA frequencies observed during the three series of tests performed right after these injections are presented in Fig. 4.

The one-way ANOVA analyzing the MA frequencies observed during the pretest revealed, as expected, no significant difference between the six groups, $F(5, 48) = 0.02$, $p = 0.9998$, because birds in the different groups had been matched on this criterion. By contrast, MA frequencies observed after injections of the DA agonists and antagonists significantly differed in each of the three different pharmacological conditions [low D_2, $F(5, 48) = 2.88$, $p = 0.0237$; high D_2, $F(5, 45) = 3.24$, $p = 0.0139$; high APO, $F(5, 41) = 4.53$, $p = 0.0022$].

To better identify the origin of these differences, the data were reanalyzed by two-way ANOVA with the first injection (V1 vs. PIMO vs. SPIP) and the second injection (V2 vs. APO) as independent factors. This revealed significant effects of both factors in each of the three series of tests. The behavior was affected by the D_2 antagonists [low D_2, $F(2, 48) = 5.55$, $p = 0.0068$; high D_2, $F(2, 45) = 5.26$, $p = 0.0088$; high APO, $F(2, 41) = 3.57$, $p = 0.0374$] as well as by the APO, except during the first series of tests [low D_2, $F(1, 48) = 3.28$, $p = 0.0764$; high D_2, $F(1, 45) = 5.85$, $p = 0.0196$; high APO, $F(1, 41) = 15.37$, $p = 0.0003$]. No interaction between the two types of treatments was, however, observed [low D_2, $F(2, 48) = 0.01$, $p = 0.9957$; high D_2, $F(2, 45) = 0.17$, $p = 0.8414$; high APO, $F(2, 41) = 0.01$, $p = 0.9963$], which clearly showed that the D_2 antagonists were unable to modify the reaction to apomorphine.

MA frequencies in the different groups were also compared two by two with the Fisher PLSD test and the corresponding results are reported in Fig. 4. In agreement with our previous findings (see Experiments 1 and 2), APO inhibited the T-induced copulatory behavior and this effect reached statistical significance at the 1-mg/kg dose. (control vs. APO: $p < 0.05$). Treatment with the D_2 antagonists alone (PIMO or SPIP) did not significantly alter the MA frequencies but a mean increase was consistently observed in birds treated with SPIP and this effect came close to significance ($p < 0.10$ vs. control) during the first series of tests (low D_2-). This moderate stimulation was also revealed by the fact that birds in the SPIP group were always significantly more active than those treated with APO alone. The inhibitory effects of APO were not affected by preinjection of pimozide and MA frequencies in the APO + PIMO group were always comparable to those observed in the APO alone group. The stimulatory effect of spiperone was still visible when this antagonist was combined with APO. During the last series of tests (high APO), the APO + SPIP birds were not, like birds treated with APO alone, significantly inhibited by comparison with controls. However, the inhibitory action of APO was still present in this condition because MA frequencies in the APO + SPIP group were significantly lower than in the SPIP alone group (see Fig. 4 for detail).

Birds in the six experimental groups had similar body weights at the start, $F(5, 48) = 0.43$, $p = 0.8246$, and at the end, $F(5, 38) = 0.78$, $p = 0.5642$, of the experiment and a small overall increase in weight was observed as is typical for quail at that age (start: 229.6 ± 27.7; end: 246.7 ± 28.9; mean ± SD). In response to the T treatment, birds developed cloacal gland areas that were not significantly affected by the dopaminergic agonist and antagonists, $F(5, 38) = 2.243$, $p = 0.0698$. The glands were substantially smaller in two of the groups that had received APO injections (APO and APO + PIMO) but none of these difference reached significance by comparison with the control group (Fisher PLSD, $p >$

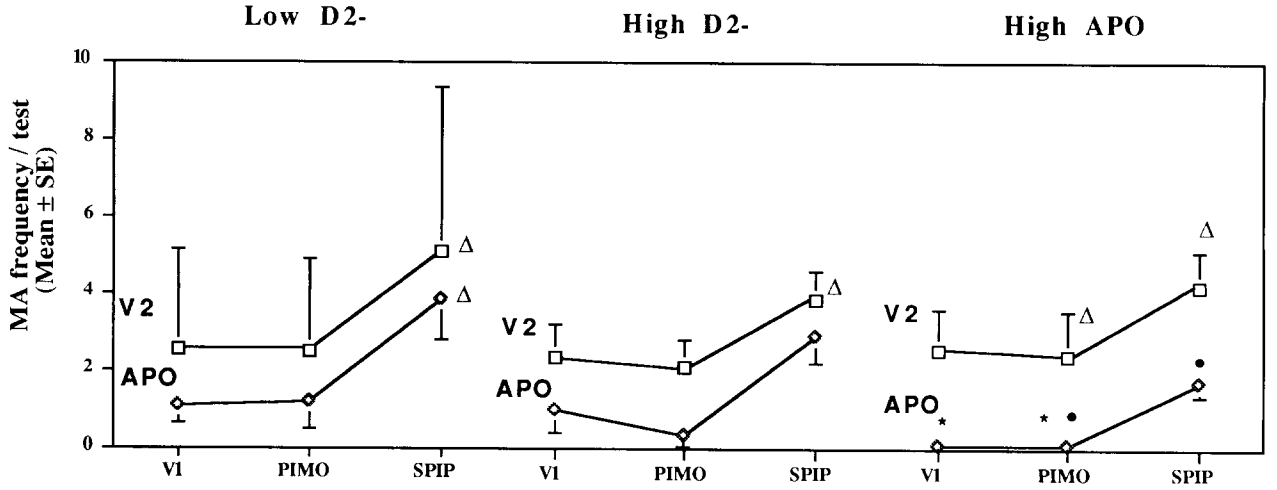

FIG. 4. Frequency of mount attempt (MA) observed in quail injected with various doses of two D_2 antagonists [pimozide (PIMO) and spiperone (SPIP)] or their vehicle (V1) in combination with apomorphine (APO) or its control vehicle solution (V2). Means and SEMs are shown. Data collected under each drug condition were analyzed by analysis of variance (ANOVA) followed by Fisher PLSD tests to compare groups two by two. The results of these statistical analyses are shown next to the data points [*$p < 0.05$ by comparison with the control (V1 + V2) group; Δ = $p < 0.05$ by comparison with the APO group; and ● = $p < 0.05$ by comparison with the corresponding group treated with the D_2 antagonist alone, that is, PIMO vs. APO + PIMO and SPIP vs. APO + SPIP).

0.05). The comparison of the mean gland size in the six groups suggested that it was somehow related to the intensity of the sexual behavior. A correlation analysis was therefore carried out to compare the mean gland size at the end of the experiment and the mean MA frequency during the last series of tests (high APO). This revealed the presence of a significant relationship ($r = 0.873, p = 0.023$; see Fig. 5).

These two variables were also related in the entire population when individual values were considered ($r = 0.586, p < 0.0001, n = 44$). A positive correlation was similarly observed within the control group ($r = 0.858, p = 0.0031, n = 9$) but not in any of the other groups that had been treated with dopaminergic agents ($r \leq 0.27, p \geq 0.50$). No correlation coefficient could be calculated in the APO and APO + PIMO groups because all birds in these groups were sexually inactive.

DISCUSSION

Neuroanatomical data based essentially on tyrosine hydroxylase immunocytochemistry and receptor autoradiography (1,8,9) suggested that DA may be implicated in the control of reproduction in quail. Three experiments during which DA agonists and antagonists were systemically injected to castrated male quail confirmed that this neurotransmitter is indeed able to profoundly modulate the T-induced male copulatory behavior in this species. The general agonist, APO, inhibited in a dose-dependent manner the behavior of males (Experiment 1). This effect was short-lived and no clear effect of repeated treatment could be detected when birds were tested 1 day after their last APO injection (Experiment 2). The last experiment suggested that dopaminergic D_2 antagonists are able to enhance T-induced copulatory behavior in quail but that they cannot block the inhibitory action of APO at this level.

Although they were induced by systemic treatments, these effects appeared specific in nature with the possible exception of the behavioral inhibition induced by the highest dose of APO (10 mg/kg) during Experiment 1. The change in male sexual behavior was in this case associated with tremors so that its physiological significance is questionable. However, because the same inhibition of MA was observed for lower doses of APO that were not associated with these side effects it is legitimate to assume that APO is able to produce a specific modulation of male copulatory behavior. No significant alteration by the dopaminergic drugs of the body weight or of the T-induced cloacal gland growth was ever detected in any of the three experiments. There was, however, a suggestion of a modulation of the CGA by some of the drugs used in Experiment 3 and this point will be discussed below. During that same experiment, a few drug-treated birds also died but no significant relationship could be established between a specific type of treatment and a higher incidence of mortality.

It must also be pointed out that these experiments were all carried out on gonadectomized T-treated quail. A modulation by DA of T secretion can not therefore be invoked to explain the behavioral effects observed because the behavior of animals was activated by constant levels of exogenous steroids.

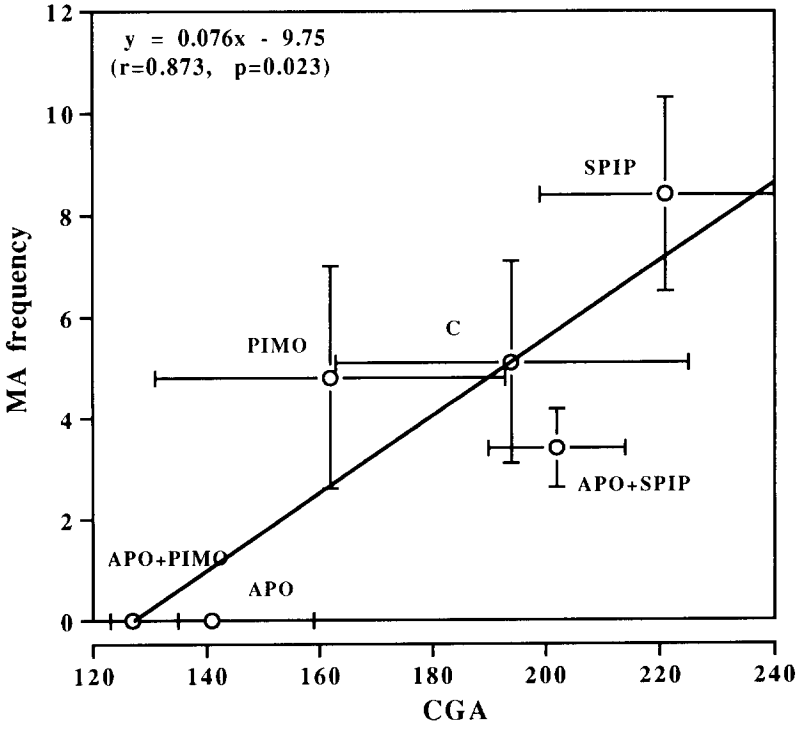

FIG. 5. Relationship between the mean sexual behavior [mount attempt (MA) frequency] observed during the last series of observations (high APO condition) and the mean cloacal gland area in the six groups of birds treated with various dopaminergic agonists and antagonists. The means and SEs of the measures have been represented and analyzed by linear regression and Pearson's product moment correlation analyses, whose results are indicated in the graph.

This contrasts with many experiments in the rat, which used gonadally intact animals. These do not permit, in theory, to differentiate between effects of DA on copulatory behavior per se from indirect effects on the pituitary-gonadal axis.

Because the Japanese quail has no copulatory organ, it is likely that peripheral effects on the genital region are of little relevance and that the central action of DA plays the most important role here. In rats, by contrast, there is evidence that the dopaminergic transmission is able to modulate both central and peripheral aspects of copulation. It is clear that the effects of the dopaminergic agonist APO on rat copulatory behavior are mediated, at least in part, by central dopaminergic receptors. APO directly injected into the preoptic area enhances male sexual behavior (21,22,53,54) and this effect is blocked by centrally acting antagonists but not by the systemic injection of domperidone, a dopaminergic antagonist that does not cross the blood-brain barrier (21,22,33,67). Part of the behavioral effects of dopaminergic agents result, however, from a modulation of penile reflexes and seminal emission achieved either by a central or local action of the transmitter (21,28,38,39,53,62,67). The central and peripheral actions of dopaminergic drugs have, unfortunately, not always been separated but they were shown, in a few instances, to be in the opposite directions (21,53,62). This problem is probably not critical in quail, which have no intromittent organ and are therefore presumably less dependent on peripheral sensory inputs.

During the present work, major inhibitory effects of IP injections of the dopaminergic agonist APO were consistently observed in quail. These effects were dose dependent over a wide range of doses (1-10,000 μg/kg) and concerned both the number of birds displaying the behavior and their activity level. In the rat, it is also well established that APO has major effects on the male copulatory behavior. In this species, however, this agonist inhibits several aspects of copulation at low doses (low μg range) but stimulates them when higher amounts (high μg to mg range) are injected. This differential action of low and high doses has been observed in the rat after peripheral as well as central (preoptic area) injections [for review, see (21,22,30,53)].

Several interpretations of this quantitative species difference can be proposed. They refer to a differential action on central vs. peripheral targets, to species differences in the dose-response curves related, for example, to a species-specific catabolism of the drugs, or to differential sensitivities of the D_1/D_2 or of the pre/postsynaptic receptors. These possibilities are briefly discussed below.

It was already mentioned that dopaminergic drugs can have opposite effects on central and genital aspects of copulation. It could therefore be speculated that the discrepancy between rats and quail results from the absence of an intromittent organ in quail. This appears, however, unlikely. Even if differential responses to dopaminergic agents have been observed in rats at the level of the central control of behavior and of the mechanisms regulating penile reflexes (see above), the specific response to a given dose of APO has always been in the same direction: Both sexual motivation and penile reflexes are inhibited by low doses of APO and stimulated by higher doses.

Alternatively, it can be assumed that the rat and the quail show similar responses to APO but that the dose-response curve is shifted in quail so that all doses tested in the present experiments still correspond to the low range that produces behavioral inhibition in the rat. In birds, the body temperature and metabolic rate are higher by comparison with rat. Steroids, for example, must always be injected in higher doses in birds than in mammals to obtain behavioral effects. If we assume that this is also the case for catecholamines, it is then possible that the highest dose of APO tested here was still in the low range for quail. Because APO was injected IP in quail and the peripheral catabolism of the drug is presumably high, it could be postulated that the amounts of the agonist reaching brain areas responsible for the control of male sexual behavior were too low to stimulate postsynaptic receptors. The treatments would preferentially stimulate autoreceptors and therefore produce the observed behavioral inhibition. It appears, however, unlikely that this interpretation alone can explain the present results. APO at the dose of 1 mg/kg fully inhibited copulatory behavior in quail. This is at least two orders of magnitude above the dose for which a switch from inhibition to activation is observed in the rat. Explaining our data on that basis would therefore require that the catabolism of APO be 100 times faster in quail than in the rat, which appears improbable. The fact that higher doses (10 mg/kg) still inhibited copulation but began to induce nonspecific effects also suggests that this interpretation probably does not reflect the reality. This means, anyway, that this interpretation cannot be experimentally tested with this kind of procedure: No specific activation of copulatory behavior with high doses of systemically administered APO will be possible because motor disturbances begin to develop before any activation is detected.

Finally, the quantitative discrepancy in the response of the rat and quail to APO may reflect a differential sensitivity of the pre- and postsynaptic (D_2/D_1) receptors in these two species. At present, a number of experimental facts support this interpretation. It can be postulated that the behavioral inhibition observed in quail treated with APO resulted from a stimulation of the D_2 autoreceptors. These are known to be 5-10 times more sensitive to DA dopaminergic agonists than postsynaptic receptors (29). Depending on the location and the functional properties of the D_2 autoreceptors, their activation induces a diminution of the dopaminergic neuronal firing rate, a reduction of DA synthesis, and/or a modification of DA release. This results in a decreased dopaminergic transmission and this could explain the subsequent inhibition of male sexual behavior.

Pharmacological studies carried out in the rat support the notion that specific activation of the dopaminergic D_2 presynaptic autoreceptors inhibits male sexual behavior (21,22,53). On one hand, D_2 agonists inhibit and D_2 antagonists stimulate aspects of the male sexual behavior. On the other hand, the intraventricular injection of a low dose of APO to male rats reduces the sexual behavior while higher doses have stimulatory effects. The inhibitory effects of the low dose are no longer observed if the dopaminergic presynaptic terminals in the MPOA are lesioned by a pretreatment with 6-hydroxydopamine (6-OHDA). Therefore, the inhibitory effects of APO appear to be linked to the presence of functional presynaptic receptors while facilitatory effects would be attributed to an action at the postsynaptic level.

In quail, some evidence was also obtained here suggesting that D_2 receptors modulate sexual behavior. Some facilitation was indeed observed after treatment with the D_2 antagonist spiperone but this was only evidenced in indirect statistical comparison (see the results of Experiment 3 for detail). Stimulation of these receptors could therefore be responsible for the behavioral inhibition observed in APO-treated animals. It is indeed established that even if APO is a mixed D_1/D_2 dopaminergic agonist this compound preferentially acts as full ago-

nist at D_2 receptors and as partial agonist at D_1 receptors (51). In addition, there is evidence based on autoradiographic experiments suggesting that the ratio of D_2 to D_1 receptors may be higher in birds than in mammals (50). If this is true, the relative increase in the number of D_2 binding sites in birds would favor the action of APO at this level and result in the inhibition of behavior observed in quail irrespective of the injected dose.

One result only is not in full agreement with this interpretation: The pretreatment of quail with D_2 antagonists such as spiperone or pimozide did not block the inhibitory effects of APO on copulatory behavior. We have, however, no independent evidence demonstrating that the doses of D_2 antagonists that were used here completely blocked all D_2 receptor sites. It has also been established that D_1 and D_2 dopaminergic receptors can develop supersensitivity after treatments with antagonists (29,34,42,46,59). Therefore, a partial blockade of the D_2 autoreceptors potentially obtained following the pretreatment with the D_2 antagonists (spiperone, pimozide) could have sensitized the remaining receptors so that the subsequent injection of APO would still be able to produce its behavioral effects by acting on a smaller number of supersensitized binding sites. The different effects of pimozide and spiperone would then be interpreted by referring to a differential affinity or specificity for the autoreceptors. Alternatively, there is recent evidence that APO is able to inhibit the in vitro DA release by mechanisms that would not be mediated by D_2 receptors but would rather involve a direct inhibition of tyrosine hydroxylase activity and therefore of DA synthesis (64). It must be acknowledged, however, that these interpretations are based on a knowledge of the DA receptors that has been obtained essentially in rats. Even in that species, the pre- vs. postsynaptic localization of the D_2 receptors has not been established unequivocally [see (27) for discussion]. It is, in addition, possible that species differences exist especially at the quantitative level. Additional pharmacological and biochemical experiments in quail using a variety of specific agonists and antagonists should therefore be performed to test the present interpretations.

In conclusion, the present experiments strongly support the notion that the dopaminergic transmission plays an important role in the control of male copulatory behavior in quail, like in mammals. The available data are consistent with the idea that increases in the postsynaptic dopaminergic tone facilitate the expression of copulatory responses but additional pharmacological work would be needed to establish the exact contribution of the different receptor subtypes. If this conclusion holds true, the sex difference in DA turnover that has been demonstrated in the quail medial preoptic nucleus could then play a key role in the control of the dramatic behavioral dimorphism: Females would never show male-type copulatory responses because they would have irreversible deficiencies in their preoptic dopaminergic system.

These observations then raise the question of how DA affects male sexual behavior. As argued recently in more detail (11), there are at least three distinct possibilities. DA could be a part of the cascade of neurochemical changes initiated by T in the brain and the T-induced increase in dopaminergic transmission would be a key factor in the activation of behavior. This interpretation is challenged by the fact that no consistent changes in DA turnover have been so far detected in the brain of rats submitted to steroid treatments that restore copulation [see (21,53) for additional discussion]. Similarly, in quail no significant change in the DA turnover within the POA or even within the POM could be detected after treatment of castrated males with T (15,49). The way in which steroids could affect the dopaminergic transmission is therefore unclear at present.

Alternatively, DA could be modulating the action of T or its metabolites (e.g., estradiol) in the brain. This idea is supported by the fact that in the mammalian brain treatments with DA agonists or antagonists modulate estrogen receptors (23,24,35,48,63) and also maybe aromatase activity through a control of cAMP (11,25,26,45,65). In quail, recent anatomical studies provide a potential substrate for such an interaction. Double-label immunocytochemistry has indeed demonstrated the presence of tyrosine hydroxylase-immunoreactive fibers in the close vicinity of aromatase-immunoreactive cells in the POA and in the area of the nucleus accumbens–stria terminalis (9). Such tyrosine hydroxylase-immunoreactive fibers are also found in association with cells containing immunoreactive estrogen receptors in many brain areas (9). In this context, it is interesting to note that during Experiment 3 a strong correlation was observed between the T-induced sexual behavior in birds submitted to treatments with various dopaminergic agents and the cloacal gland growth in these different groups of birds. This may suggest that the DA agonist and antagonists had general effects on the physiology of subjects but, alternatively, it is also conceivable that these drugs affected the action of T not only in the brain (behavioral effects) but also on the cloacal gland. One problem in this interpretation is that the behavioral action of T depends on its aromatization and subsequent binding of the locally produced estrogens to estrogen receptors (10,14,16,20) while the cloacal gland growth is activated by androgens acting as such (3,6,7,17). We have data indicating that the dopaminergic system is able to modulate aromatization and estrogen binding (see above) but so far there is, to our knowledge, no indication that DA could modulate androgen action. However, because the binding of DA to its receptors modulates the production of cAMP (29,42), which is a widespread intracellular second messenger, it is conceivable that androgen action may also be affected by DA. This effect was, admittedly, of limited amplitude here because no significant difference in cloacal gland size was observed between groups (these glands were simply correlated with the behavior) but it certainly indicates that this type of action should receive further attention.

Finally, it is also conceivable that the dopaminergic transmission and the steroids act independently on separate mechanisms that converge subsequently to result in the behavioral activation. Most of the research carried out until recently has assumed more or less implicitly that the first of these hypotheses (T acting on DA activity) was correct. Recent anatomical evidence points, however, to the second of these possibilities (DA modulating T action). Further research on this fundamental problem is currently under progress in our laboratory.

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