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STIMULATORY EFFECTS OF THE NORADRENERGIC NEUROTOXIN DSP4 ON SEXUAL BEHAVIOR IN MALE QUAIL

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ABSTRACT

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Four separate experiments were carried out to test in castrated male Japanese quail the effects on testosterone-induced sexual behavior of the neurotoxic drug N-(2-chlorethyl) -N- ethyl -2-bromobenzylamine hydrochloride (DSP4). In each experiment, DSP4 enhanced some aspects of the testosterone-induced sexual behavior. The latency between the start of testosterone treatment and the first occurence of copulatory behavior was decreased, the behavior frequency and duration were increased, and the latency between the introduction of a stimulus female and the first sexual behavior was decreased. The effects could be reversed by concurrent treatment with the alpha-adrenergic agonist, phenylephrine. The stimulatory effects of DSP4 on behavior were not seen in castrated birds which did not receive a concurrent treatment with testosterone and they were associated with a significant decrease of the norepinephrine concentration in the hypothalamus. These results suggest that the noradrenergic system plays an inhibitory role in the control of copulatory behavior of quail similar to what has been described in the ring dove.

KEY WORDS: sexual behavior, DSP4, testosterone, Japanese quail

INTRODUCTION

That monoamines play an important role in the regulation of male reproductive behavior has long been established (for review: Meyerson and Malmnäs, 1978; Meyerson and Eliasson, 1978; Crowley and Zemlan, 1981). In rats, pharmacological studies consistently point to a stimulatory effect of dopaminergic transmission on male copulatory behavior (Malmnäs, 1973; Tagliamonte et al., 1974; McIntosh and Barfield, 1984a). The role of central noradrenergic systems is by comparison poorly understood. A number of studies carried out in rats suggest however that norepinephrine (NE) stimulates, in this species, some aspects of copulatory behavior. Injection of yohimbine (an α-2 receptor antagonist) increased mounting performance (Clark et al., 1984) while treatment with clonidine (α-2 agonist) inhibited copulatory events in rats (Meyerson et al., 1979). The blockade of α-2 receptors, which are in part located presynaptically, should result in a

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potentiation of the postsynaptic actions of endogenous norepinephrine caused by the attenuation of negative feedback mechanisms (see Clark et al., 1984). These pharmacological manipulations thus suggest that endogenous NE potentiates sexual behavior.

In addition, lesions of the medial forebrain bundle which are associated with a major depletion of telencephalic NE concentrations impaired male copulatory behavior of rats (Caggiula et al., 1973) and electrolytic lesion of the *locus coeruleus* (origin of many noradrenergic fibers) increased the duration of their post-ejaculatory refractory period (McIntosh and Barfield, 1984b). In the same study, depletion of NE concentrations by synthesis inhibitors also increased mount and intromission latencies and the length of the refractory period. Recently, it was also found that DSP4 which kills noradrenergic neurons, decreases sexual behavior in rats (prolonged ejaculatory latencies and post-ejaculatory intervals: Hansen et al., 1982). All these data thus support the notion that NE stimulates male sexual behavior but a number of negative reports have also been published throwing some doubt on the generality of this finding (see Meyerson and Eliasson, 1978, Meyerson and Malmnäs, 1978 or Meyerson et al., 1979 for review). In addition, a number of studies also point to a possible inhibitory role of NE, at least over some aspects of male copulatory behavior (see Crowley and Zemlan, 1981).

Few data are available concerning the behavioral implications of noradrenergic transmission in birds. In the ring dove, Barclay and Cheng (1984) showed that systemic treatment with phenylephrine (an alpha adrenergic agonist) specifically decreases the incidence of bow-cooing (aggressive component of courtship). The adrenergic antagonist, prazosin had opposite effects. More recently the same authors confirmed this inhibitory role of NE on bow-cooing in a number of drug experiments using central administration of noradrenergic drugs (Barclay and Cheng, 1985, 1986).

Sexual dimorphism in copulatory behavior has long been established in Japanese quail. Gonadectomized males show copulatory behavior in response to testosterone treatment while females do not (Adkins and Adler, 1972; Adkins, 1975; Balthazart et al., 1983; Schumacher and Balthazart, 1983). It was shown recently that this behavioral sex dimorphism corresponds to a neurochemical brain dimorphism: the concentration of norepinephrine (NE) in the preoptic area (POA) is higher in females than in males (Ottinger et al., 1986) and the brain turnover of NE is affected differently in males and females by gonadectomy and testosterone replacement therapy (Ottinger and Balthazart, 1987). Knowing that NE inhibits some aspects of reproductive behavior in ring dove (see above), it is then tempting to speculate that the higher concentration of NE in the POA of females is responsible for their behavioral insensitivity to testosterone. To support this idea, it is however necessary to study which role, if any, plays the noradrenergic transmission in the control of copulatory behavior in quail. We are presently engaged in a series of experiments designed to study this problem by treating birds with various agonists and antagonists of the noradrenergic system and analyzing the

behavioral effects of these treatments. In this paper, we report the results of four experiments using a relatively specific noradrenergic neurotoxic: the DSP4 or N-(2-chlorethyl) -N- ethyl -2-bromobenzylamine hydrochloride. In rats, systemic injection of this drug produces a long lasting depression of NE concentrations in the cerebral cortex and other parts of the brain without any or only minor alterations of the dopaminergic and serotonergic neurons (Jonsson et al., 1981; Ross, 1976; Hallman et al., 1984). Similar effects have been described in the young chick (Davies et al., 1985).

MATERIAL AND METHODS

Experimental animals

Male quail (*Coturnixcoturnixjaponica*) were obtained from a local breeder. Birds were gonadectomized at the age of 3-4 weeks under hypnodil anesthesia (15 mg/kg of body weight, Janssens Pharmaceutica, Belgium) and then raised in groups without hormone treatment under a long photoperiod (16L:8D; light on at 0600 h) until the age of sexual maturity (6-7 weeks). A few days before the beginning of the hormone treatment (day one), they were isolated in individual cages under the same photoperiod. The mean body weight of the animals used in all experiments was slightly above 200 g. Food (commercial flour for egg laying hens) and water were always available *ad libitum* .

Experimental procedures

During experiment 1 and 2 before any treatment, birds were tested for of sexual behavior (pretest). Only males which were sexually inactive during this test were used during the experiment. Males were randomly assigned to experimental groups and on day one were implanted with 3 (experiment 1 and 2) or 2 (experiment 3 and 4) silastic capsules (20 mm each; Dow Corning Silastic tubing number 602-252; 1.57 mm i.d., 2.41 mm o.d.) filled with testosterone (T; Sigma T-1500) or left empty as control. On that same day (experiment 1, 2 and 3) or one day before (experiment 4), birds in some groups also received a single i.p. injection of DSP4 or N-(2-chlorethyl) -N- ethyl -2-bromobenzylamine hydrochloride (batch 151-15a generously supplied by Astra Lakemedel, Södertalje, Sweden) at a dose of 25 or 50 mg/ kg body weight or a control injection of saline solution (NaCl 0.9 %). DSP4 was dissolved in the saline solution at 50 mg/ml. On several occasions before and during the experiments, birds were weighed and their cloacal gland area (an androgen-dependent structure) was measured with a caliper (greatest length x greatest width). At sacrifice, completeness of castration and presence of the implants were controlled. Data from birds with incomplete castration or in which implants could not be found were discarded before any analysis.

Behavioral tests

Throughout the experiment, male sexual behavior was regularly tested in standardized conditions. A stimulus female was introduced into a test arena (59 x 38 x 43 cm height) and thirty seconds later, the male was added. Latencies and frequencies of the following behavior patterns were recorded during the next 5 min: neck-grab (NG), mount attempt (MA), mount (M) and cloacal contact movements (CCM; see Adkins and Adler, 1972 or Hutchison, 1978 for a detailed description of these behaviors). Similar conclusions could be drawn in the analysis of these different measures and summaries of the behavioral data will only be presented to avoid redundancy. During the 5 minutes of test, we also recorded the number of minutes during which sexual behavior was observed at least once. This generated a score (presence-absence) of 0 to 5 which will be referred to as occurence score in the following.

On several occasions (12 and 7 times in experiments 1 and 2 respectively), birds were also tested for the occurence of crowing. To determine the crowing rate, we counted in the home cages the number of crows performed by each bird in the course of a 5 min period. Quail were observed in a random order in groups of 8 during different moments of the lights-on portion of the light-dark cycle.

Cumulative percentages of birds showing a given behavior were analyzed by Fisher exact probability tests. Behavioral frequencies and occurence scores were analyzed by Kruskal Wallis

analyses of variance followed by paired comparisons with the Mann Whitney U-test. Morphological data were analyzed by one way analyses of variance (ANOVA) followed by Newman Keuls tests.

Monoamines assays

During experiment 4, the effects of DSP4 on the brain concentration of monoamines was directly evaluated using HPLC and electrochemical detection. Four days after the injection of DSP4, birds were sacrificed by decapitation and their brain was quickly dissected out of the skull and frozen on dry ice. The following part were then dissected for the assay: left telencephalon (TEL; 131.64±13.92 mg, mean±S.D.), medial and posterior hypothalamus (HYP; 14.61±3.53 mg), left optic lobe (OL; 65.93±11.56 mg), cerebellum (CER; 59.14±17.67 mg) and brain stem (BS; 65.36±15.35 mg).

The concentration of monoamines (norepinephrine [NE], epinephrine [E], dopamine [DA], and serotonin [5-HT]) was assayed in these selected brain areas by high performance liquid chromatography (HPLC) combined with electrochemical detection (ECD). The chromatographic system consisted of a Rheodyne Inc. model 7125 sample injector, an LKB HPLC pump (model 2150), a guard column (4x30 mm) and a chromatography column (4x100 mm) both filled with Nucleosil 100-5 C18 (Macherey Nagel, Düren, FRG), and an LKB electrochemical detector (Model 2143). Chromatograms were recorded either by a Roche Bio-electronics pen recorder in peak height mode at 100 mV full scale deflection or were digitized by a specially designed A/D converter, stored on Apple 2e disks and later integrated by means of a specific software (Chromlab-III) designed by Alltech-RSL (Belgium). Mobile phase was prepared in the following way: 2.84 g Na_2HPO_4 (0.02M) were dissolved into one liter of water/methanol (75:25), pH was adjusted to 3.5 with concentratedH_3PO_4, and 0.007% sodium dodecylsulfate were added. The mobile phase was then filtered and degassed over 0.45 µm filters. Flow rate was set at 1.0 ml/min and oxidation potential on the detector at 400 mV.

Stock solutions of norepinephrine (NE), epinephrine (E), dopamine (DA), serotonin (5-HT), and dihyroxybenzylamine (DHBA) were prepared at a concentration of 100 µg/ml in 0.05 M $HClO_4$ containing 5 mM $Na_2S_2O_5$. Immediately before the assay, these stocks were diluted in 0.08 M acetic acid to produce standards at the concentration of 1 ng/20µl (=the injection volume).

Brain samples were homogenized in 0.08 M acetic acid at a concentration of 40 or 50 mg/ml and centrifuged for 15 min at 15,000 g. Before homogenization, DHBA was added to the samples in order to correct results for losses during the extraction procedure and variations in injection volume or detector sensitivity. Supernatants were collected and stored in a freezer at -70°C until assayed. The pellets were redissolved in 1N NaOH and assayed for protein content by the method of Bradford (1976). The mean protein content of the different brain areas was between 10 and 15% (TEL: 14.09±2.70; HYP: 10.11±0.74; OL: 15.18±1.33; CER: 11.87±2.12; BS: 10.08±1.15). Twenty µl fractions of the supernatants were directly injected in the HPLC system.

For each set of samples, the system was calibrated by injecting known amounts (usually 1 ng) of the amines and of the internal standard (DHBA). The peak heights (or peak areas if the Apple 2e based integration system was used) for the different compounds were determined and the ratios amines/DHBA were calculated. The same measurements and calculations were then made for the actual samples. The concentrations of amine per sample was obtained by dividing the ratio for the sample by the ratio for the standard, multiplying the result by the amine content in the standard and finally dividing by the amount of tissue (fresh weight) or of protein equivalent to the sample (Internal standardmethod).

We have demonstrated that the detector response (peak height) of the detector is linear in the range 100 pg to 10 ng for all compounds considered. The parallelism of the assay for quail brain was established by comparing the amounts of NE, E, DA, and 5-HT detected in the same brain homogenate assayed at different dilutions. For this experiment, a pool of quail hypothalami was homogenized at a concentration of 20 mg/ml. Dilutions were then made at 15, 10, and 5 mg/ml and assayed for their amine content (assay on 20 µl i.e. 400, 300, 200 or 100 µg fresh weight). The concentrations of amines expressed per mg fresh weight were not affected by the dilution of the sample. Accuracy of the technique was determined by addition of increasing known amounts (0, 100, 200, 400, or 800 pg) of NE, E, or DA to a brain pool. The quantities measured were always highly correlated with the quantities added ($r = 0.985$ or better in each case) and the slope of the regression of measured versus added quantities was not different from 1 (except in the case of NE where a slight underestimation was detected) thus indicating that amines are accurately measured

when diluted in a brain homogenate. Reproducibility of the assay was evaluated by measuring 8 times the same hypothalamic sample or the same standard at 1 ng/20µl. The coefficient of variation (CV) for the different amines varied between 5 and 12 percent except in the case of E in the hypothalamus (CV=19.5%) because the concentration of the compound was too low (CV for E in the standard was 8.02%). This assay method can thus be considered as parallel, accurate, and reproducible for the measurement of monoamines in the quail brain. It can easily detect amine quantities in the 100 pg range which means that measurements can be taken on brain areas of 0.1 mg or less.

RESULTS

Experiment 1

During this experiment, we tested the effects of DSP4 at the dose of 25 or 50 mg/kg body weight on the testosterone-induced sexual behavior. Four groups of castrated males were selected and injected on day one with DSP4 25 mg/kg (group DSP4-25, n=7), DSP4 50 mg/kg (group DSP4-50, n=6) or with saline solution (group T, n=8 and group C, n=6). On the same day, the first 3 groups (DSP4-25, DSP4-50 and T) were implanted subcutaneously with 3 capsules filled with testosterone while the control (C) group received 3 empty capsules. Sexual behavior was tested on every other day between day 3 and 17 after implantation. Cloacal gland area and body weight were measured on days 4, 8, 12 and 16. Results of the behavioral tests are summarized in figure 1.

As expected on the basis of previous studies, T activated copulatory behavior in all castrated males in less than 2 weeks. The simultaneous treatment with DSP4 increased the effects of T. The behavioral difference by comparison with control birds became significant after a shorter latency (3 days instead of 7), the behavioral frequencies reached higher levels and occurence scores were higher in DSP4 treated birds than in birds receiving only T. These effects of DSP4 are reflected in the statistical analysis by higher levels of significance in the comparisons with the control group and in some case by significant differences in the comparison with the T group (see figure 1 for detail). These effects were more pronounced for the lowest dose of DSP4.

Crowing frequency was increased in T-treated birds by comparison with controls but DSP4 did not enhance the effects of T on this response (median total frequencies were 0, 4.5, 1 and 15 in the groups C, T, DSP4-25 and DSP4-50 respectively; groups T and DSP4-50 are significantly different from group C by Mann Whitney U test for $2p<0.05$). Similarly T induced a dramatic increase in cloacal gland area but DSP4 had no clear additional effect at this level. On day 16, cloacal gland areas were 20.5, 293.6, 317.2 and 270.2 mm^2 in groups C, T, DSP4-25 and DSP4-50 respectively. In the course of the experiment, the DSP4-25 group was never significantly different from T group; the DSP4-50 group had smaller glands than the T group on day 4 and had smaller glands than DSP4-25 group on days 4, 8 and 12).

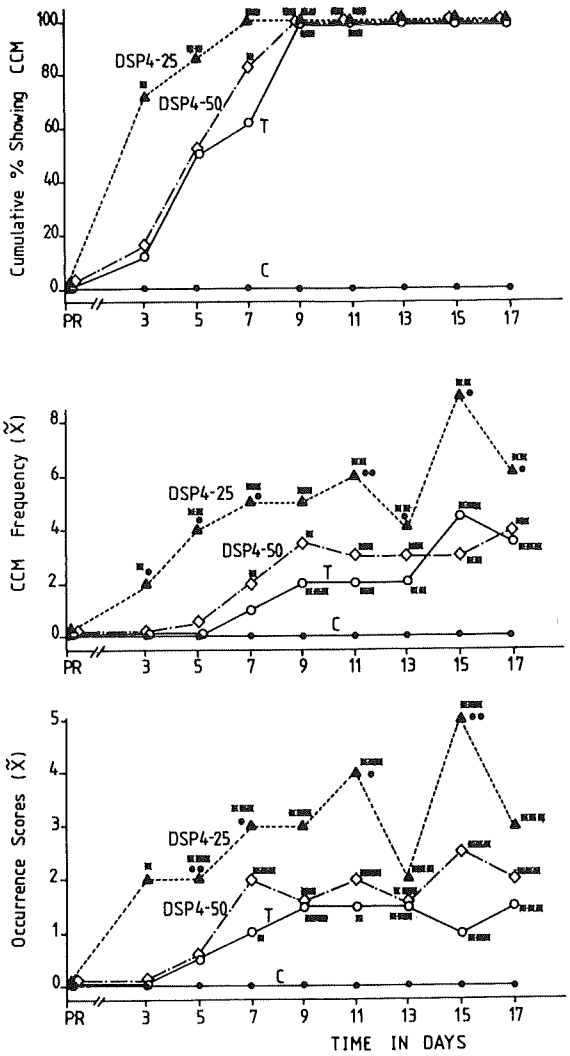

Fig. 1. Sexual behavior of castrated male quail treated with testosterone (T) or with T and DSP4 at 2 different doses (25 or 50 mg/kg) or maintained without treatment as control (C). The 3 panels show from top to bottom respectively the cumulative percentage of males who showed cloacal contact movements (CCM), the median frequency of CCM and the occurence scores of sexual behavior (see text for definition). Data were analyzed by Fisher tests or Mann Whitney U-tests following significant Kruskal Wallis ANOVA. *= $2p<0.05$, **= $2p<0.01$, ***= $2p<0.001$ for comparisons with the C group; •= $2p<0.05$, ••= $2p<0.01$ for comparison with the T group.

Experiment 2

This second experiment was undertaken to study the reproducibility of the behavioral effects of DSP4 and study whether these could be reversed by treatment with a noradrenergic alpha-agonist, phenylephrine. It is indeed established that DSP4 causes a degeneration of noradrenergic neurons and fibers but in the long term probably leaves more or less intact the postsynaptic receptors (Jonsson et al., 1981; Dunwiddie et al., 1983). If the behavioral effects observed in experiment 1 were the result of the reduced noradrenergic transmission, then injection of a noradrenergic agonist should adequately counteract the action of DSP4. It had been previously demonstrated that phenylephrine inhibited some aspects of reproductive behavior in ring doves (Barclay and Cheng, 1984, 1985, 1986). In addition, the same authors had demonstrated that the noradrenergic regulation of reproductive behavior in ring dove only implicated the α receptors and that β adrenergic drugs such as isoproterenol and propranolol were without effect. This justified the selection of phenylephrine as adrenergic agonist in the present experiment.

Five groups of castrated males were selected and, on day one, 4 of them were implanted with 3 capsules filled with testosterone while the fifth one received empty capsules as control. Before this on the same day, two of the testosterone-treated groups had received a single injection of DSP4 at the dose of 25 mg/kg in saline (25 mg/ml). Other birds received an injection of saline solution. Starting on day one, two of the groups were in addition injected twice a day for 8 days with phenylephrine (L-phenylephrine hydrochloride, Sigma, St Louis, Mo) at a dose of 15 mg/kg in each injection. The daily injections were given i.p. around 9.00 a.m. and 6.00 p.m. Five groups were defined in this way: controls (C, n=6), testosterone (T, n=8), testosterone + DSP4 (DSP4, n=9), phenylephrine (PE, n=6) and testosterone + DSP4 + phenylephrine (DSP4+PE, n=9). Between days 1 and 8, the birds which were not injected with phenylephrine were injected in the same conditions with a saline solution as a control. Injections of PE were discontinued on day 8 to test for the reversibility of PE effects but also because birds receiving this treatment showed signs of toxicity (see below). Sexual behavior was tested repeatedly during 15 days after the start of the treatments. Test were performed at randomly selected hours during the day that is one to 8 hours after the first PE injection. Cloacal gland and body weight were measured on days 1, 8 and 16.

Behavioral results obtained in this experiment are summarized in figure 2. Like in the previous experiment, DSP4 enhanced the effects of T on sexual behavior. This is reflected again by a faster appearance of copulatory behavior after implantation with T, by the higher frequency of CCM and the higher occurence scores. In the statistical comparisons, results of the DSP4 group are more different from the C group than results of the T group and in addition, in several cases the DSP4 and T groups are significantly different (see figure 2 for detail). These stimulatory effects of DSP4 were almost completely suppressed by concurrent treatment with PE.

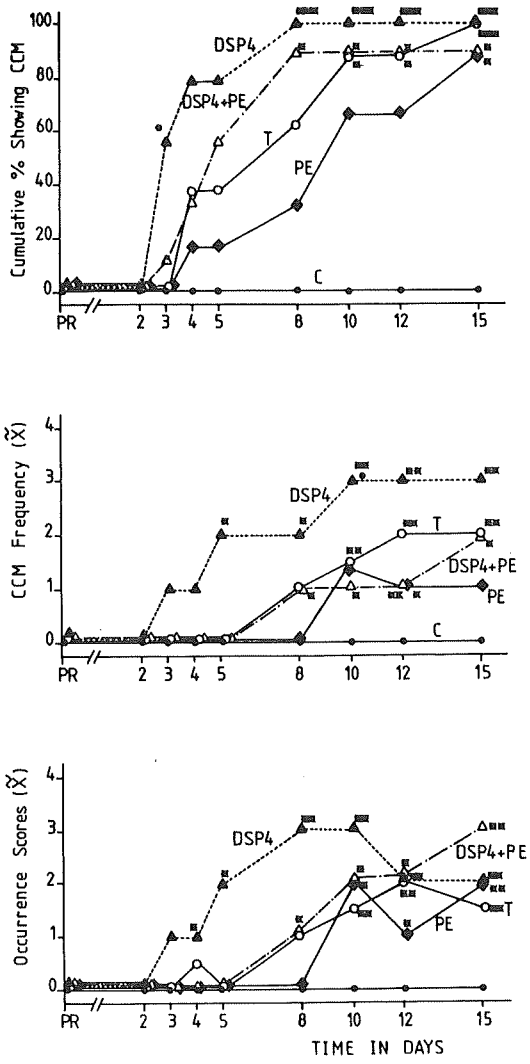

Fig 2. Sexual behavior of castrated male quail treated with testosterone (T), DSP4, phenylephrine (PE) or receiving combined treatments or left intacts as control (C). See legend of figure 1 for additional details.

In addition, PE alone (without DSP4) decreased the behavioral response to T and slowed down the appearance of copulatory behavior. The interpretation of these effects of PE is however complicated by the fact that, on diferent days, variable durations elapsed between the injection of the agonist and the behavioral test. The observed effect could thus result from the adrenergic receptor

stimulation but also from short- and long-term adaptative receptor changes. Additional work should be carried out to discriminate between these possibilities.

Crowing frequency was increased in all groups receiving T implants. By comparison with C birds, the crowing frequency was significantly higher in the group T (median frequency during the whole experiment= 1.5), DSP4 (median frequency= 2) and DSP4+PE (median frequency= 1) but not in the group PE (median frequency= 1.5; absence of significant difference is however only related to the smaller size of this group).

Cloacal gland area was significantly increased in the 4 groups bearing T implants by comparison to the C group ($p<0.01$ by Newman Keuls test for each group on days 8 and 16). These 4 T-treated groups were not significantly different except for the DSP4+PE group on day 8 whose glands were significantly smaller than in the other 3 groups ($p<0.05$, Newman Keuls test).

Body weights were similar in the 5 experimental groups on days 1 and 16 (ANOVA with $p>0.10$; mean±S.D. on day 16: 211±24). However on day 8, the ANOVA of body weights indicated significant differences ($F=4.90$, $p<0.004$). Newman Keuls tests showed that birds in the DSP4+PE group and in the PE group were lighter than birds in the T group. Body weights of other groups were not significantly different.

Experiment 3

The first two experiments had shown that sexual behavior in T-treated castrated quail appeared more rapidly and was more intense when birds had been treated simultaneously with the noradrenergic drug, DSP4. A third experiment was then undertaken to research whether DSP4 had behavioral effects on its own or if its action was contingent on the presence of T. Male quail were castrated at the age of three weeks and two weeks later were randomly assigned to one of 4 groups which were either implanted with T silastic capsules (2 x 20 mm) and/or injected with DSP4 (50 mg/kg). Control treatments were the implantation of empty silastic capsules and/or injection of saline. This resulted in 4 groups treated with T alone (group T, n=11), T and DSP4 (T+DSP4 group, n=12), saline alone (S group, n=6) or saline and DSP4 (S+DSP4 group, n=7). Sexual behavior of the birds was observed on day 3 and 6 after the start of the treatments. Birds were sacrificed on day 7.

Behavioral results are summarized in table 1. Like in previous experiments, behavioral activation was faster and more pronounced in birds receiving simultaneous treatment with T and DSP4 than in birds receiving T alone. No effect of DSP4 was detected when the birds did not at the same time receive a treatment with T. One bird in the S+DSP4 group was sexually active on day 3 but no longer on day 6 but this is probably only because castration was still fairly recent and we have, on occasion, seen birds retaining sexual behavior for periods as long as 2 weeks after

gonadectomy (no pretest had been performed here).

GROUPS		T	T+DSP4	S	S+DSP4
% Showing	NG	64 *	92***	0	14
	MA	64 *	83 **	0	14
	M	46	83 *	0	14
	CCM	46	83 *	0	14
Behavior Frequency (Median, Range)					
	NG	3 *	3 **	0	0
		(0-11)	(0-13)	(0-0)	(0-1)
	MA	2 *	2.5 *	0	0
		(0-10)	(0-9)	(0-0)	(0-1)
	M	0	1 *	0	0
		(0-8)	(0-7)	(0-0)	(0-1)
	CCM	0	1 *	0	0
		(0-5)	(0-5)	(0-0)	(0-1)

Table 1: Percentage of castrated birds showing sexual behavior and frequency of the different behavior patterns after treatment with testosterone (T) and/or DSP4 or after the control treatments. All data are relative to the sum of the two tests performed on days 3 and 6. Behavioral scores have been compared with those of the saline group by the Fisher exact probability test (percentages) or by the Mann Whitney U-test (frequencies). * = $2p<0.05$, ** = $2p<0.01$ and *** = $2p<0.001$.

Experiment 4

In the third experiment, DSP4 had been shown to enhance T-activated sexual behavior at the dose of 50 mg/kg. This last experiment was designed to test the reproducibility of this effect and, at the same time, evaluate the effect of DSP4 on the brain concentrations of monoamines. Male quail were castrated between 3 and 4 weeks of age and 10 days later were injected with DSP4 at the dose of 50 mg/kg dissolved in saline (50 mg/ml). Control birds (S group) were injected with saline. On the next day, the DSP4-treated birds and some of the saline birds received two 20 mm silastic implants of T while the others birds of the S group received two empty implants of the same length. This generated 3 groups of birds: S group (n=12), T group (n=18) and T+DSP4 group (n=12). The sexual behavior of the birds was tested on day 4 after DSP4 injection and they were sacrificed on day 5. Brain concentrations of norepinephrine (NE), epinephrine (E), dopamine (DA) and serotonin (5HT) were assayed in 7 birds in each group.

GROUPS		T	T+DSP4	S
% Showing	NG	33	67***	0
	MA	33	58 **	0
	M	17	42 *	0
	CCM	6	33	0
Behavior Frequency (Median, Range)				
	NG	0	1.5 *	0
		(0-5)	(0-6)	(0-0)
	MA	0	1 *	0
		(0-3)	(0-3)	(0-0)
	M	0	0	0
		(0-4)	(0-3)	(0-0)
	CCM	0	0	0
		(0-1)	(0-2)	(0-0)

Table 2: Percentage of birds showing sexual behavior after treatment with testosterone (T) combined or not with DSP4 or after the control treatments (S). Behavioral scores have been compared with those of the saline group by the Fisher exact probability test (percentages) or by the Mann Whitney U-test (frequencies). * = $2p<0.05$, ** = $2p<0.01$ and *** = $2p<0.001$.

Table 2 shows the percentage of birds which displayed the different types of sexual behaviors in the 3 experimental groups. Like in the previous experiment, DSP4 enhanced the behavioral effects of T and this despite of the slightly different experimental protocol.

The concentrations of monoamines measured in the brain of these birds are presented in the table 3. As can ben seen, DSP4 decreased the NE concentration in all brain areas but this effect was usually of small amplitude and reached significance only in the hypothalamus (25% decrease compared to the T group). No other significant effect of the treatments (T or DSP4) was detected except in the brain stem where concentrations of 5HT were decreased by T treatment with no additional effect of DSP4. The injection of DSP4 also tended to reduce E concentrations in the hypothalamus and 5HT concentrations in the hypothalamus and telencephalon but none of these effects was significant.

DISCUSSION

The four experiments presented in this paper show that one single injection of the noradrenergic neurotoxin, DSP4 has long-lasting effects on the copulatory behavior of castrated male quail. More specifically, the DSP4 treatment decreased the latency between the implantation of testosterone and the first occurence of cloacal contact movement, it increased the frequency of this behavior and also, in general, the occurence scores of sexual behavior.

AMINE	AREA	T	T+DSP4	S	F	p
NE	HYP	36.73±5.03	27.62±6.61 *	34.19±6.50	4.16	*
	TEL	5.05±0.94	4.17±1.59	6.29±4.27	1.09	ns
	CER	3.05±1.06	2.18±0.75	2.69±1.22	1.10	ns
	OL	4.06±0.79	3.45±1.21	4.32±0.66	1.53	ns
	BS	6.63±1.90	5.55±1.01	7.92±2.71	2.45	ns
E	HYP	10.60±4.51	5.97±2.84	8.47±2.88	3.06	ns
	TEL	-	-	-	-	
	CER	-	-	-	-	
	OL	-	-	-	-	
	BS	-	-	-	-	
DA	HYP	7.18±1.35	6.28±2.51	7.58±3.24	0.50	ns
	TEL	10.57±3.63	7.48±0.75	9.38±1.61	3.12	ns
	CER	-	-	-	-	
	OL	0.25±0.32	0.22±0.30	0.40±0.42	0.48	ns
	BS	0.95±0.93	1.01±1.28	1.78±1.26	1.08	ns
5HT	HYP	5.31±3.02	3.64±1.18	4.32±2.86	0.79	ns
	TEL	11.46±2.71	8.63±2.73	12.71±4.93	2.34	ns
	CER	-	-	-		
	OL	4.75±0.88	5.45±2.80	6.65±2.73	1.20	ns
	BS	7.31±1.41	7.75±2.26 ψ	10.15±1.73 *	4.83	*

Table 3: Concentration (ng of amine/mg protein) of norepinephrine (NE), epinephrine (E), dopamine (DA) and serotonin (5HT) in the hypothalamus (HYP), telencephalon (TEL), cerebellum (CER), optic lobe (OL) and brain stem (BS) of castrated male quail treated with testosterone (T), testosterone and DSP4 (T+DSP4) or with saline (S). Data were analyzed by one way ANOVA (F values and probabilities in the two rightmost columns) followed by Newman Keuls tests when appropriate. * (ψ) = $p<0.05$ compared to the T (S) group. Data are not reported when only traces of the amines were present and could therefore not be measured accurately.

This result has in addition been confirmed during two other experiments performed in our laboratory and is thus quite reliable. It is important to note that these results were observed using different experimental protocols including different doses of the drug, different treatments with T and different modalities of behavior tests. The amplitude of the behavioral effects of DSP4 was limited. It is an enhancement of the effect of T not an all-or-none phenomenon. It was however demonstrated in each experiment by the fact that the behavior of the T+DSP4 birds was always more frequent and more intense that the behavior of the birds treated with T alone. The limited amplitude of the effect made it difficult to obtain a significant behavioral difference between birds treated with T alone and

with T and DSP4 but the reality of this effect is attested by its reproducibility. In most experiments however the number of birds which showed copulatory behavior (CCM) within 5-6 days after T implantation was about doubled when a concurrent treatment with DSP4 was applied which represents a biologically relevant effect. If we pool the results of the 4 experiments presented here and consider the number of birds showing CCM within 6 days of the start of T treatment (cumulative percentage of active birds on day 5 for experiment 1 and 2, day 6 for experiment 3 and day 4 in experiment 4), a very significant effect of DSP4 is detected (CX: 0 birds out of 30 show CCM and this score increases to 13 out of 45 for T birds and 28 out of 46 for T+DSP4 birds; the difference T *versus* T+DSP4 is significant for p=0.0043 by the Chi-square test).

DSP4 alone was unable to activate copulatory behavior in castrated birds which did not receive a concurrent treatment with T. The presence of T thus seems to be required in order to observe sexual behavior and DSP4 is not able to substitute for the steroid. Considering that other androgen dependent response such as crowing or growth of the cloacal gland are not modified by the DSP4 treatment, it can be stated the stimulation of testosterone-induced copulatory was quite specific. This demonstrates that the effects of DSP4 are not simply a consequence of a decreased peripheral catabolism of testosterone or of a general activation/sensitization of the androgen receptors. The fact that crowing behavior is not affected by DSP4 also suggests that the drug only acts on specific regions of the brain. In the experiments presented here, sexual behavior was measured in castrated birds whose sexual behavior was activated by silastic implants of T . This rules out an interpretation of the effect of the drug which would be based on a modification of the secretion of the activating hormone.

By contrast to the effects observed here, inhibitory effects of DSP4 on male sexual behavior have been reported in rats: several days after an injection of 50 mg/kg of DSP4, ejaculatory latencies and post-ejaculatory intervals are significantly increased (Hansen et al., 1982). In that experiment, sexual behavior was however evaluated in intact animals and copulatory behavior was activated by the endogenous testosterone. It is thus difficult to evaluate whether the inhibition of behavior by DSP4 was caused by a central effect of the drug rather then by a decrease of testicular secretions.

It is extremely likely that the behavioral effects of DSP4 result from its neurotoxic noradrenergic properties. The assay data presented in experiment 4, show that, following DSP4 treatment, the concentration of only one monoamine was significantly decreased in only one brain area: NE concentrations were significantly reduced in the hypothalamus. Similar trends were also observed in the other brain regions but they did not reach significance. The available data thus point to a major action of the drug on the hypothalamic noradrenergic system. It is however also interesting to note here that treatments with T significantly reduced the 5HT concentration in the brain stem. In mammals, it is generally accepted that 5HT inhibits copulatory behavior(e.g. Meyerson and Malmnäs, 1978; Meyerson et al., 1979). The decrease in 5HT concentrations observed here

following T treatment could thus be a part of the mechanisms by which the steroid activates reproductive behavior. This idea should now be experimentally tested.

In rats, DSP4 causes a long term decrease in NE concentration in many brain regions although cortical areas are always the most affected (Ross, 1976; Jaim-Etcheverry and Zieher, 1980; Jonsson et al., 1981; Hallman et al., 1984; Logue et al., 1985). Similar effects have been observed in one avian species, the domestic chick in which they were associated with behavioral changes (Davies et al., 1985). The effect of DSP4 on the NE system is quite specific and usually leaves intact other neurotransmitters or neuromodulators such as dopamine, serotonin, adrenaline, acetylcholine, GABA, glycine or aspartic acid (Jacobson and Wilkinson, 1985). To be complete, it must nevertheless be mentioned that a number of studies have identified in some brain areas unspecific effects of DSP4 such as increased dopamine concentration (Hallman et al., 1984), decreased dopamine concentration (Jonsson et al., 1981), decreased serotonin concentration (Hallman et al., 1984; Meert, 1985), or interaction with the opiate receptors (Jacobson and Wilkinson, 1985). However, the amplitude of these neurochemical modifications is always small or they are observed only a short time after the DSP4 injection or they are not easily reproducible. In the present experiment, there was similarly some indication that other aminergic systems could have been affected by the DSP4 (small decreases in DA and 5HT) as reported previously in mammals (see above). However none of these effects reached significance with the sample size which was used. When compared to the larger long-lasting depletion of norepinephrine, it seems unlikely that these additional effects of DSP4 are responsible for the behavioral effects observed here. The assay data obtained here support if they do not prove this interpretation.

During experiment 2, it was also possible to suppress the stimulatory effects of DSP4 on copulatory behavior by simultaneous injections of the alpha adrenergic agonist, phenylephrine. It has been shown that in rats, electrophysiological responses to the direct action of beta- and alpha-adrenergic agonists are unchanged following DSP4 treatment which suggested that the DSP4-induced norepinephrine depletion is not necessarily associated with postsynaptic changes in the adrenergic system (Dunwiddie et al., 1983). It is thus possible that the treatment with phenylephrine suppressed the effects of DSP4 by restoring at the postsynaptic level the noradrenergic inputs which had been suppressed by the drug. Changes in receptors sensitivity should also be considered due to the variable interval between the injection of the drug and the behavioral test. In the PE alone group, the inhibition of behavior would then be the result of a supranormal noradrenergic activity. This interpretation is supported by the fact that the PE-dependent inhibition of copulatory behavior was reversible and essentially disappeared after the end of the injections (after day 8). However the behavioral inhibition could also result from non-specific toxic effects as suggested by the reduced body weight gain, smaller cloacal glands and presence of ulcerations at the injection site in some

birds. This should be tested by the use of alpha-adrenergic antagonists and by more detailed dose-response studies. It must however be pointed out that in ring doves, PE also inhibits aspects of the reproductive behavior. The behavioral effects can be observed following systemic injection but also after infusion into the third ventricle and opposite effects are obtained by treatment with the alpha-adrenergic antagonist, prazosin (Barclay and Cheng, 1984; Barclay and Cheng, 1985). These observations support the idea that behavioral inhibition by PE is a specific phenomenon. If this conclusion is accepted, then the results of this experiment demonstrate that the noradrenergic transmission inhibits male sexual behavior in quail, that this effect is mediated by alpha receptors (probably α-1) and that these receptors are not destroyed following treatment with DSP4.

The site of action of these pharmacological manipulations remains unclear at present. In mammals, it is clear that DSP4 causes a preferential degeneration of noradrenergic nerve terminal projections originating from the locus coeruleus (e.g. cerebral cortex, hippocampus) and has more limited effects on the hypothalamic noradrenergic innervation (Ross, 1976; Jonsson et al., 1981). The assay data presented here point to a larger effect in the hypothalamus although the existence of a localized depletion of NE in some telencephalic regions cannot be ruled out.

In quail, the concentrations of norepinephrine are by far higher in the hypothalamus than in any other brain region (Ottinger et al., 1986; present data). However significant concentrations are present in the telencephalon and their turnover is controlled by steroids (Ottinger and Balthazart, 1987). In addition, recent in vitro quantitative autoradiographic studies (Balthazart et al., 1987; Gahr et al., 1987) demonstrate the presence of high concentrations of α-1 adrenergic receptors in areas such as the neostriatum caudale, the archistriatum ventrale, the cortex piriformis and the cortex dorso-lateralis while major localizations of α-2 adrenergic receptors are the preoptic area-hypothalamus but also the hyperstriatum ventrale, the septum lateralis and the archistriatum ventrale (see Bayle et al., 1974 for the nomenclature of brain areas). The role of these different areas containing noradrenergic receptors is not clear at present and their participation in the control of copulatory behavior is not established. The participation of preoptic and hypothalamic systems is by far the most likely. In the preoptic area, we have identified a nucleus (the medial preoptic nucleus or POM) whose volume is sexually dimorphic (Viglietti-Panzica et al., 1986). This volume decreases after castration and increases back to normal levels after testosterone treatment (Panzica et al., 1987). On the neurochemical side, it has been shown that the POM contains high levels of aromatase activity (an enzyme which transforms T into estradiol, a steroid which is supposed to play a key role in the activation of behavior; Schumacher and Balthazart, 1987) and this enzymatic activity is also testosterone-dependent (decreased by castration and increased by testosterone; Schumacher and Balthazart, unpublished data). The POM contains in parallel high concentrations of estrogens receptors as shown by immunocytochemistry (Balthazart et al., 1987) and *in vitro* autoradiographies

have shown that it contains very high levels of α-2 adrenergic receptors (Balthazart et al., 1987). The POM thus appears as an important site of neuroendocrine interactions. Considering that the preoptic area is a major brain site for the control of male copulatory behavior in quail (Watson et al., 1986; Balthazart and Surlemont, unpublished data) like in other birds and mammals (Kelley and Pfaff, 1978), it seems possible that the neuroendocrine interactions which take place in POM could be related to the control of reproductive behavior. In this context, it is interesting to note that in one study it was found that NE concentrations were higher in the preoptic area of females than males (Ottinger et al., 1986). If the noradrenergic transmission really plays an inhibitory role on sexual behavior, the higher adrenergic activity in females could then contribute to explain the fact that it is not possible to induce copulatory behavior in females by testosterone treatments (see introduction). Experiments are now in progress to test this possibility.

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