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Effects of Δ^9-Tetrahydrocannabinol on Copulatory Behavior and Neuroendocrine Responses of Male Rats to Female Conspecifics

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MURPHY, L. L., J. GHER, R. W. STEGER AND A. BARTKE. *Effects of Δ^9-tetrahydrocannabinol on copulatory behavior and neuroendocrine responses of male rats to female conspecifics.* PHARMACOL BIOCHEM BEHAV **48**(4) 1011–1017, 1994.—Male rats exposed to sexually receptive females, exhibit a rapid increase in plasma levels of luteinizing hormone (LH) and prolactin, and concomitant increases in noradrenergic activity in the medial basal hypothalamus (MBH) and median eminence (ME) as well as in dopaminergic activity in the MBH. Delta-9-tetrahydrocannabinol (THC; 5 mg/kg b.wt., PO), the chief psychoactive constituent of marijuana, blocked the MBH and ME noradrenergic response and the dopaminergic response in the MBH in male rats exposed for 20 min to sexually receptive females, and suppressed the expected increases in plasma LH and prolactin levels. Moreover, THC treatment decreased the percentage of animals exhibiting copulatory behavior and increased the latency periods to mount and intromit. These findings indicate that THC interferes with the neuroendocrine and behavioral responses of male rats to the presence of a receptive female.

Delta-9-tetrahydrocannabinol Copulatory behavior Male sexual arousal Luteinizing hormone Prolactin Norepinephrine Dopamine Serotonin

DELTA-9-TETRAHYDROCANNABINOL (THC), the principal psychoactive component of marijuana, inhibits many aspects of reproductive function in humans and experimental animals [for review, see (34)]. In the male rat, acute exposure to THC results in the suppression of basal luteinizing hormone (LH) and prolactin release from the anterior pituitary (36,55,56) and inhibition of testicular testosterone secretion (55,56). Evidence suggests that the ability of THC to inhibit reproductive hormone release is primarily due to an action of the drug within the central nervous system, altering the activity of neurotransmitter and neuropeptide systems that modulate, either directly or indirectly, anterior pituitary hormone secretion (36,55). Indeed, THC-induced alterations in neurotransmitter activity may be involved in the ability of THC to affect a variety of neuroendocrine and behavioral indices (4,29,46).

Although the effects of THC on male copulatory behavior have been studied, the mechanism of its action is unclear. In an early study, acute THC treatment was shown to decrease sexual motivation in the male rat (33). In male mice, copulatory behavior parameters were significantly suppressed by high doses of acute (13,51) or chronic THC treatment (14). Moreover, perinatal THC exposure disrupted copulatory behavior in adult male mice (14) and rats (17).

In many species, exposure of a male to a sexually receptive female results in dramatic changes in hypothalamic catecholamine activity and a rapid release of LH into the circulation (19,23,25,54). Studies have suggested that this rise in LH is related to sexual motivation because it is absent or severely attenuated in animals that show no interest in the female (24,25,54). Recently, it has been shown that deficits in both the arousal and performance components of male sexual behavior in experimentally induced hyperprolactinemic (54) and diabetic male rats (19) are accompanied by suppression of acute changes in hypothalamic noradrenergic transmission and LH secretion that normally occur in the male in response to a female conspecific.

The present experiments were designed to determine the effects of THC on the neuroendocrine and hormonal responses of male rats exposed to a sexually receptive female.

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These findings will provide new information on the neuroendocrine consequences of THC exposure and relate these findings to reproductive behavioral effects of THC in the male.

METHOD

Animals

Adult Sprague-Dawley rats [Hsd : Sprague-Dawley (SD)BR] were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and were subsequently maintained in air-conditioned, constant-temperature rooms (23 ± 1°C) on a 12L : 12D alternating artificial light schedule (lights off at 1800 h). Food and water were supplied ad lib. Six weeks prior to testing, female rats were ovariectomized and immediately implanted with a 5 mm Silastic capsule (0.125 in o.d. × 0.095 in i.d.) filled with 17β-estradiol (Sigma). Approximately 5 h before testing, female rats were injected sc with 0.5 mg progesterone (Sigma). Just prior to experimentation, receptivity of each female rat was tested using additional male rats not used for data collection, and only those females exhibiting the lordotic posture were used for testing.

Experimental Protocol

All experiments were performed between 1830 and 2130 h, starting 30 min after lights were turned off, in an area dimly lit by 20 W red bulbs. Male rats were given a single oral dose of THC (5 mg/kg) or sesame oil vehicle. THC of greater than 95% purity was provided in an ethanol solution by the National Institute on Drug Abuse. Immediately before use, THC was added to a sesame oil vehicle (Sigma) and the alcohol was evaporated under nitrogen at 45–50°C to achieve the desired dose (5 mg/kg b.wt.) in a volume of 0.1 ml.

Response to female conspecifics. Forty minutes after treatment, half of the THC-treated ($n = 16$) and half of the vehicle control rats ($n = 16$) were individually placed in cages containing a receptive female. The remainder of the animals treated with THC ($n = 16$) or vehicle ($n = 16$) were also removed from their home cages, but were individually placed in empty cages without a female. To allow measurement of neurotransmitter activity within different regions of the CNS, half of the animals in each group received an injection of a tyrosine hydroxylase inhibitor, α-methylparatyrosine (α-MPT; 250 mg/kg, IP), 30 min after treatment with THC or oil vehicle for the determination of catecholamine turnover rates. After 20 min of exposure to a female rat or an empty cage (i.e., 60 min after THC or vehicle and 30 min after injection of saline or α-MPT), the male animals were sacrificed by decapitation for plasma and tissue collection. Trunk blood was collected from non-α-MPT-treated animals into centrifuge tubes containing 6% EDTA (anticoagulant) in saline and plasma was prepared and stored frozen for the analysis of LH, prolactin, and testosterone. The median eminence (ME) was removed with iridectomy scissors using magnification prior to freezing the brain on dry ice and storing all brain tissue at −75°C for subsequent dissection and amine determinations.

Behavioral analysis. Animals to be used in tests for copulatory behavior were subjected to three preliminary mating tests of 30-min duration. Only animals that achieved intromission (mounting with pelvic thrusting and penile insertion) within 15 min, and ejaculation within 15 min of the initial intromission in at least two trials were utilized. One week after the third trial, the rats were treated with either THC (5.0 mg/kg b.wt., PO; $n = 10$) or vehicle ($n = 10$) and 25 min later were placed in bedding-lined 10-gallon glass aquaria. Each animal was allowed 5 min to acclimate to the test chamber before the introduction of a sexually receptive female. The following parameters were recorded: mount latency (ML), time from introduction of the female until first mount with pelvic thrusting; intromission latency (IL), time from introduction of the female until the first mount with pelvic thrusting and vaginal penetration; ejaculation latency (EL), time from the first intromission until ejaculation; postejaculatory interval (PEI), time from ejaculation until next intromission; mount frequency (#M), number of mounts prior to ejaculation; intromission frequency (#I), number of intromissions prior to ejaculation. Copulatory testing was terminated after the postejaculatory interval was recorded or at 15 min if an intromission had not been registered.

Radioimmunoassays

Plasma LH, prolactin, and testosterone concentrations were determined in single radioimmunoassays using assay procedures described in our previous publications (9,35,38). The assay sensitivities were 0.024, 0.0195, and 0.025 ng/ml, and the intraassay coefficient of variation was 6.3, 4.4, and 3.4% for LH, prolactin, and testosterone, respectively. Gonadotropin results were expressed in terms of the NIADDK rat LH or prolactin reference preparation RP-3.

Neurotransmitter Determinations

Norepinephrine and dopamine content in the median eminence, medial basal hypothalamus (with the median eminence removed), and anterior hypothalamic/preoptic area, and serotonin (5-HT) and 5-hydroxyindole-3-acetic acid (5-HIAA) concentrations in the medial basal hypothalamus were measured by high performance liquid chromatography with electrochemical detection as previously described (52). Briefly, ice-cold 0.1 N $HClO_3$ containing methyl serotonin and dihydroxybenzylamine as internal standards for the indoleamine and catecholamine assays, respectively, was added to every tissue sample. Each sample was sonicated and an aliquot of this mixture was centrifuged at 12,000 × g for 1 min for the serotonin assay. Additionally, an aliquot of this mixture was prepared for the catecholamine assay by alumina extraction (52).

Data Analysis

Hormone and neurotransmitter data were analyzed using two-way analysis of variance to determine the statistical significance ($p < 0.05$) of differences between treatment means. The percentage of males mounting, intromitting, and ejaculating in control vs. THC-treated animals was evaluated nonparametrically with Fisher's exact probability test. Latency periods and number of mounts or intromissions preejaculation in vehicle vs. THC-treated animals were evaluated with the t-test for independent samples, since these parameters were not always registered for all animals. Only those animals with recorded mounts, intromissions, or ejaculations were included.

RESULTS

Exposure to a receptive female led to a significant elevation of LH and prolactin levels ($p < 0.05$) in male rats when compared to nonexposed controls (Fig. 1). The administration of THC significantly reduced LH levels ($p < 0.05$) in peripheral plasma but did not significantly affect plasma prolactin levels (Fig. 1). The expected increases in LH and prolactin levels

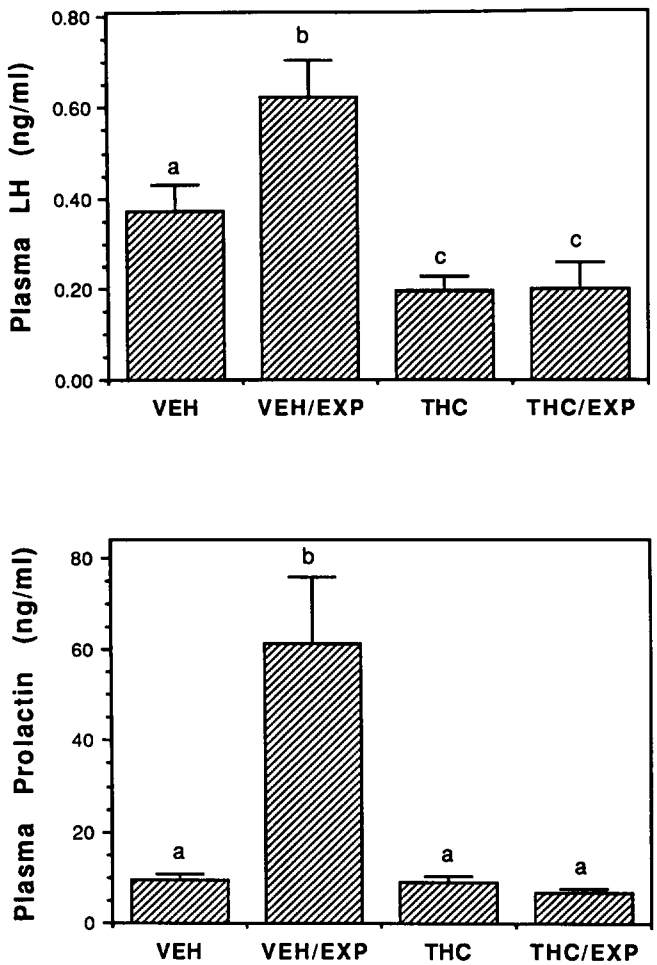

FIG. 1. Effects of a 20-min exposure (EXP) to a sexually receptive female rat on concentrations of plasma LH and prolactin in control (VEH) or delta-9-tetrahydrocannabinol-treated (THC) male rats (mean ± SEM). Male rats were treated with either sesame oil vehicle or THC (5 mg/kg b.wt., PO) 40 min prior to female exposure (n = 7–8 animals per group). Letters above each bar denote significance at $p < 0.05$ when compared to bars with different letters.

after female exposure were completely suppressed in male animals treated with THC. It was particularly striking that the very pronounced increase in plasma prolactin levels observed in female-exposed control rats was totally absent in female exposed-THC-treated rats even though basal prolactin levels in this group were not altered. Testosterone levels remained unchanged in male rats 20 min following female exposure and/or after THC treatment when compared to controls (Fig. 2).

Exposure of control (vehicle-treated) males to sexually receptive females produced significant increases ($p < 0.05$) in median eminence (ME) and medial basal hypothalamic (MBH) norepinephrine turnover and MBH dopamine turnover (Fig. 3). A single oral dose of THC significantly decreased both ME and MBH noradrenergic activity when compared to vehicle controls ($p < 0.05$). Although THC suppressed dopamine turnover within the ME, MBH dopamine turnover was not affected by THC treatment. Moreover, within the ME, female exposure reversed the inhibition of dopamine turnover produced by THC (Fig. 3). Neither the content of 5-HT and 5-HIAA, a major metabolite of serotonin, nor the ratio of 5-HT/5-HIAA, which provides a measure of serotonin metabolism, were altered by female exposure or THC treatment, suggesting that THC did not affect hypothalamic serotoninergic systems (Fig. 4). There were no consistent or significant changes in norepinephrine or dopamine turnover rates in the anterior hypothalamus/preoptic area of the male brain following female exposure or THC treatment (data not shown).

Treatment with THC 30 min prior to behavioral testing significantly decreased the proportion of males that mounted, intromitted, and ejaculated ($p < 0.05$, Table 1). Moreover, THC treatment significantly increased ($p < 0.05$) the latency to both mount and intromit. Neither ejaculation latency nor the number of mounts and intromissions preejaculation in THC-treated animals was different when compared to animals receiving vehicle. In those animals that did ejaculate, THC did not alter the latency period between ejaculation and the first intromission postejaculation.

DISCUSSION

The present studies confirm previous observations concerning the adverse effects of acute THC exposure on reproductive functions in the male rat (5,37) and provide new information concerning the ability of THC to block the neuroendocrine response of the male to exteroceptive stimuli originating from the female. Upon exposure to a sexually receptive female rat, males treated with THC secreted significantly less LH and prolactin relative to vehicle controls. The rapid increase of LH that occurs in the male exposed to a sexually receptive female has been used successfully as an index of sexual arousal (18,23). Our data suggest that THC-treated males, who display marked reductions in sexual behavior (i.e., increased mount latency), may be less sexually motivated as manifested by their significantly diminished neuroendocrine response to

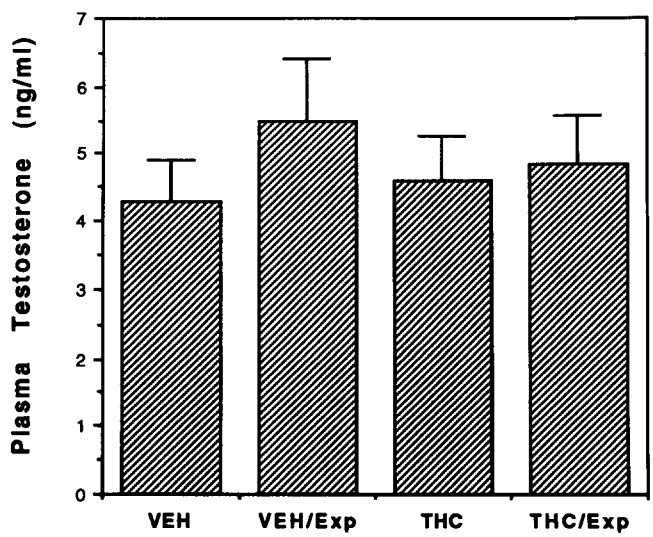

FIG. 2. Effects of a 20-min exposure (Exp) to a sexually receptive female rat on testosterone concentrations in control (VEH) or delta-9-tetrahydrocannabinol-treated (THC) male rats (mean ± SEM). Male rats were treated with either sesame oil vehicle or THC (5 mg/kg b.wt., PO) 40 min prior to female exposure (n = 7–8 animals per group).

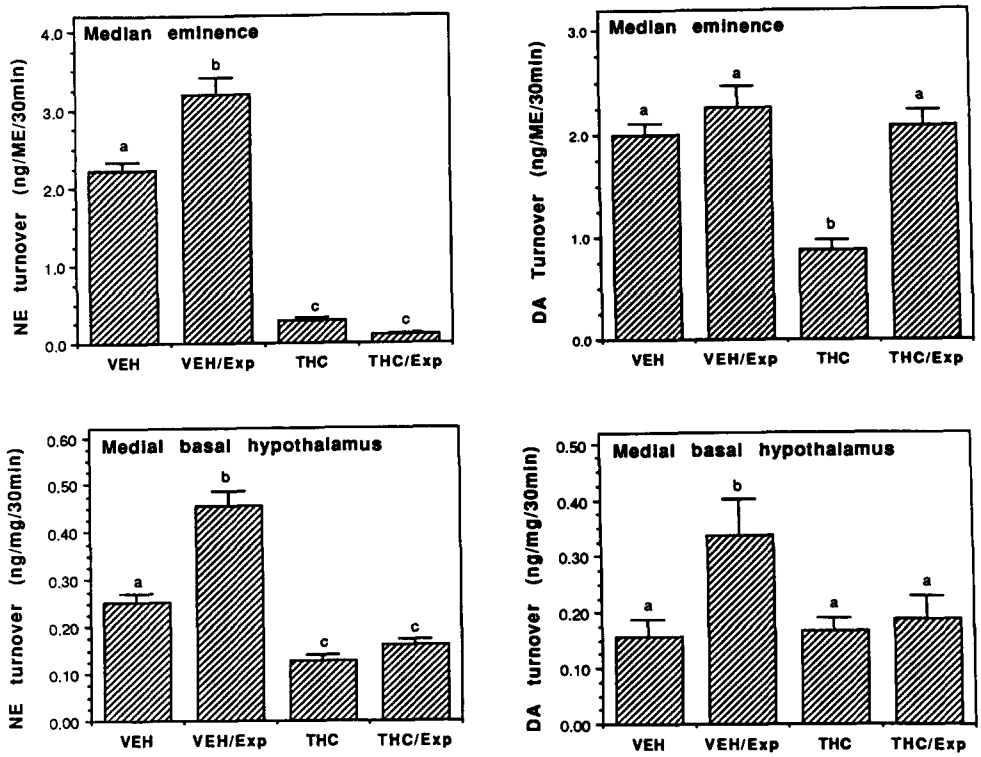

FIG. 3. Effects of female exposure (Exp) on norepinephrine (NE) and dopamine (DA) turnover (mean ± SEM; n = 7-8) in the medial basal hypothalamus and median eminence of control (VEH) or THC-treated male rats. NE or DA turnover, an index of neuronal activity, was calculated from the decline of NE or DA content after inhibition of tyrosine hydroxylase with α-MPT. Letters above each bar denote significance at $p < 0.05$ when compared to bars with different letters.

the sexually receptive female. Hence, the decrease in LH release may provide a measure of neuroendocrine abnormalities responsible for the dramatic reduction in copulatory behavior exhibited by males acutely exposed to THC.

The ability of acute THC administration to inhibit the release of LH in intact animals and inhibit the pulsatile fluctuation in LH release in the gonadectomized animal has been well documented (7,31,37,43,58). Recently, we demonstrated that the microinfusion of THC (0.1-10 μM) into the median eminence and specific brain nuclei located within the medial basal hypothalamus inhibited pulsatile LH secretion in a dose-related manner (16). This suggests that THC suppresses LH secretion through some alteration of the central neuroendocrine control of the pituitary, presumably inhibition of gonadotropin-releasing hormone (GnRH) release into the hypothalamic-hypophysial portal system. Indeed, hypothalamic GnRH concentrations are increased following THC treatment in ovariectomized rats (52,61), and the administration of GnRH reverses the inhibition of LH release by THC in experimental animals (2,57). The MBH/ME region is the site of terminal fields of noradrenergic neurons that provide major stimuli to GnRH-releasing neurons and, thus, indirectly control the release of LH (22). A number of studies have demonstrated that changes in norepinephrine turnover are often associated with altered LH secretion (3,53,54). In our studies, cannabinoids have been shown to have pronounced effects on the noradrenergic system. The administration of a low dose of THC (0.5 mg/kg b.wt., PO) in intact male rats significantly reduced NE turnover in the median eminence at 30 and 60 min after THC administration and significantly decreased plasma LH levels 60 min after THC treatment (37). Hypothalamic dopamine activity and serotonin concentrations were not af-

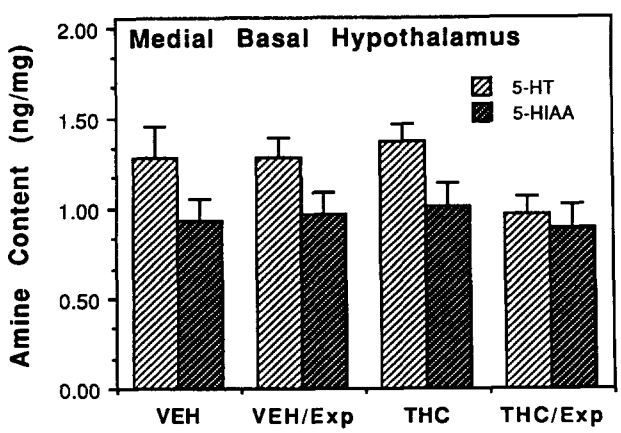

FIG. 4. Effects of female exposure (Exp) on serotonin (5-HT) and 5-hydroxyindole-3-acetic acid (5-HIAA) content in the medial basal hypothalamus in control (VEH) or THC-treated male rats.

TABLE 1

SEXUAL BEHAVIOR SCORES FROM ANIMALS TESTED
60 MIN AFTER RECEIVING SESAME OIL VEHICLE OR THC (5.0 mg/kg b.wt., PO)

Group	*n*	%M	%I	%E	ML*	IL*	EL*	PEI*	#M*	#I*
vehicle	10	100	100	100	80.7 ± 17.3†	271.4 ± 55.6	1184.0 ± 180.1	485.8 ± 20.1	28.6 ± 5.1	9.7 ± 1.0
THC	10	50‡	30‡	30‡	565.6 ± 126.7‡	703.9 ± 103.6‡	1281.3 ± 205.9	478.0 ± 13.3	30.0 ± 8.0	12.3 ± 1.9

Columns provide the number of animals (*n*) in each treatment group, percentage of males that mounted (%M), intromitted (%I) or ejaculated (%E) as well as mean mount latency (ML), intromission latency (IL), ejaculation latency (EL), and latency to the first postejaculatory intromission (PEI), and the mean number of mounts (#M) and intromissions (#I) prior to the first ejaculation during a 30-min test. Mount, intromission, ejaculation, and postejaculatory intromission latencies are in seconds.

*Only animals for which these parameters actually were registered are included.

†Values are reported as mean ± SEM

‡$p < 0.05$ vs.the respective vehicle control.

fected by THC administration. Moreover, intracerebroventricular administration of NE stimulated LH release in castrate, testosterone-primed male rats pretreated with THC (36). These data support the concept that the inhibitory effects of THC on peripheral LH levels are secondary to THC-induced alterations in NE activity and subsequent inhibition of GnRH secretion. Thus, the inability of THC-treated male rats to respond to female conspecifics with an LH surge may be due to reduced activity of noradrenergic neurons within the medial basal hypothalamus and median eminence of these animals, and particularly to suppression of the normal response of these neurons to stimuli originating from the females (49,54).

Particularly striking was the robust increase in prolactin levels that occurred in males 20 min after exposure to a sexually receptive female and the finding that THC pretreatment suppressed this prolactin surge. Earlier studies had demonstrated rapid, yet transient, increases in both LH and prolactin concentrations in the plasma of sexually naive male rats physically exposed to a stimulus female (23,24). Although THC itself did not alter basal plasma prolactin levels in this study, it has previously been shown to decrease (27,44,45), increase (44,59), or have no effect (8) on prolactin secretion, depending on the animal model and dose and mode of THC administration. Prolactin release appears to be controlled primarily by inhibitory dopaminergic inputs to the anterior pituitary (30). Although THC does have effects on dopaminergic neurotransmission (4,11,40,47), which have been linked to the effects of THC on irritable aggression (47), locomotor activity (46), and euphorigenic properties (10), a connection between THC-induced changes in dopaminergic activity and corresponding changes in prolactin secretion is less clear (39,45). In the current study, female exposure stimulated hypothalamic DA turnover and increased plasma prolactin levels. Furthermore, THC treatment in nonexposed male rats reduced ME DA turnover but had no effect on plasma prolactin levels. The possibility that a) dopaminergic activity is altered more immediately after THC administration or female exposure, or b) the prolactin surge in female-exposed male rats is a direct result of other neurotransmitters or neuropeptides known to affect the release of prolactin cannot be dismissed at this time. Moreover, it is not clear from this study if the female-induced prolactin surge is responsible for the increase in MBH dopaminergic activity, as prolactin itself is capable of stimulating dopamine turnover via a well-described feedback mechanism (50). Recent findings that THC augments dopamine-induced inhibition of prolactin secretion in hemipituitary incubations in vitro (38,45), suggesting a direct pituitary site of THC action, also deserve consideration.

In vivo brain microdialysis (21,41,42) and ex vivo biochemical studies (32) have provided evidence that dopamine activity increases in the nucleus accumbens, striatum, and preoptic area upon exposure of the male rat to a sexually receptive female and during copulation. These brain sites appear to be important in the regulation of the arousal and performance aspects of sexual behavior in the male (28). Pharmacological stimulation of dopamine receptors activates copulatory behavior in male rats (20,48), whereas dopamine receptor antagonists impair male rat copulatory behavior (60). Pharmacological manipulations of the noradrenergic system have demonstrated that stimulation of α_1 and blockade of α_2 adrenoceptors also facilitate copulatory behavior in the male (12). In the present study, the ability of THC pretreatment to increase the latencies to mount and intromit may be due to suppression of catecholaminergic activity, as reflected by the decreases in MBH NE and dopaminergic activation. These findings suggest that the MBH may be another important brain site involved with the sexual motivation component of copulatory behavior. In our study, there were no catecholaminergic changes in the medial preoptic area (MPOA) of sexually naive male rats pretreated with vehicle or THC and exposed to a sexually receptive female. However, there is increasing evidence that the MPOA may have important influences on the performance aspect of male sexual behavior (28,48). We measured neurotransmitter activities 20 min after initial female exposure, which may not have provided adequate time to detect catecholamine changes in these sexually naive animals. Moreover, acute THC treatment appeared to impair male sexual motivation (i.e., increased mount and intromission latencies; decreased percentage of animals initiating copulatory behavior), but did not alter either the ejaculation latency or the number of mounts and intromissions preceding ejaculation in those animals that did copulate.

Plasma testosterone levels do not appear to be altered as a result of female exposure in sexually naive rats, although testosterone levels were stimulated in sexually experienced animals (23,24). In accordance with these findings, there were no changes in testosterone concentrations following female exposure in our study. Moreover, testosterone levels were not altered 60 min after THC administration although there was a significant decrease in LH release. Cannabinoids, including THC, have been reported to depress testosterone levels in vivo (26,56) and in vitro (6,15) in experimental animals and man.

It is probable that either the acute suppression in LH levels was not sufficient to decrease testosterone release or that testosterone levels were altered at a time not measured in the current study.

In conclusion, changes in the hypothalamic noradrenergic and dopaminergic transmission and in pituitary LH and prolactin release can be viewed as physiological markers of the perception of female conspecifics and of sexual arousal in the male. Thus, THC, the principal psychoactive component of marijuana, appears to be able to interfere with CNS functions leading to sexual arousal and/or with the ability of perception of sexually relevant stimuli to alter hypothalamic and pituitary function. The disruption in copulatory behavior by THC measured in this and other laboratories (33) may, therefore, reflect an impairment in the motivation of the male to engage in sexual activity. There is considerable evidence that the ability of THC to alter pituitary hormone secretion is due primarily to an action within the CNS with few if any effects exerted directly at the adenohypophyseal level (36,44,61). Therefore, we view the effects of THC on hypothalamic noradrenergic and dopaminergic neurotransmission demonstrated in the present study as a more proximal, if not the primary, event, with the effects on the pituitary being secondary to this and other CNS changes. Further work is needed to determine whether suppression of norepinephrine and dopamine turnover by THC is, indeed, responsible for its ability to interfere with male sexual behavior and whether the present results, obtained in rats, may apply also to other species.

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