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European Journal of Pharmacology 578 (2008) 37–42
www.elsevier.com/locate/ejphar

Gonadal hormone modulation of the behavioral effects of Δ^9-tetrahydrocannabinol in male and female rats

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Received 20 June 2007; received in revised form 23 August 2007; accepted 10 September 2007
Available online 19 September 2007

Abstract

Female rats are more sensitive than males to many behavioral effects of cannabinoids. The purpose of the present study was to determine if sex differences in the antinociceptive and motoric effects of Δ^9-tetrahydrocannabinol (THC) are due to activational effects of gonadal steroid hormones. THC-induced antinociception (tail withdrawal, paw pressure tests) and motoric effects (horizontal locomotion, catalepsy) were compared in male and female gonadectomized rats that were chronically treated with hormone (testosterone in males, estradiol in females) vs. those that were gonadectomized and had no hormone replacement. THC's effects were also compared between gonadally intact females tested during vaginal estrus vs. diestrus. THC (5 and 10 mg/kg i.p.) produced very similar antinociceptive effects in no-hormone vs. testosterone-treated males, but significantly less locomotor suppression in testosterone-treated males than those with no hormone replacement. In gonadectomized females, estradiol enhanced THC's antinociceptive but not motoric effects. In gonadally intact, cycling females, 5 mg/kg THC produced slightly to significantly greater behavioral effects in estrous than in diestrous females. These results suggest that sex differences in THC-induced behavioral effects in the adult rat can be attributed to activational effects of testosterone in males and/or estradiol in females.

Keywords: Sex differences; Analgesia; Catalepsy; Locomotor activity; Cannabinoid; Estrogen; Testosterone

1. Introduction

Sex differences in behavioral and physiological effects of cannabinoids have been demonstrated in adult rats. For example, several cannabinoids have been shown to be more potent or to produce greater effects in females than males in decreasing locomotor activity, and in producing antinociception, catalepsy and hypothermia (for review, see Craft, 2005).

Gonadal steroid hormones influence many aspects of neurobiology and behavior, during development and in adulthood. Traditionally, gonadal steroid effects on neurons and behavior have been divided into two categories: organizational and activational. Organizational effects are those leading to permanent sexual differentiation in the central nervous system during development, whereas activational effects are considered impermanent and are the result of hormones activating (or inhibiting) already existing circuits in the adult organism. Sex differences in mammalian behavior (or in a drug effect on behavior) generally have been found to rely on organizational and/or activational effects of gonadal steroid hormones (Becker et al., 2005).

The extent to which sex differences in behavioral effects of cannabinoids may be explained by the differential gonadal hormone milieu in adult male vs. female rodents is not yet known. However, gonadal hormones such as estradiol are known to regulate cannabinoid receptor density (Rodriguez de Fonseca et al., 1993), transcription (Gonzalez et al., 2000) and signal transduction (Mize and Alper, 2000) in some parts of the adult rodent brain. The purpose of the present study was to determine to what extent sex differences in behavioral effects of cannabinoids in the rat may be explained by activational effects of two primary gonadal steroid hormones: testosterone in males and estradiol in females.

2. Materials and methods

All protocols were approved by the Washington State University Institutional Animal Care and Use Committee (protocol #3085).

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doi:10.1016/j.ejphar.2007.09.004

2.1. Subjects

Male and female Sprague–Dawley rats, 3–4 months old, were used (bred in-house from Taconic stock, Germantown, NY). Rats were housed in same-sex pairs or triplets (females) under a 12:12 h light:dark cycle. Access to food and water were ad libitum except during surgery and testing.

2.2. Apparatus

For the warm water tail withdrawal test, a 2.5-l water bath (Precision Scientifics, Chicago, IL) with the temperature set at 50.0±0.5 °C was used. For the paw pressure test, an Analgesymeter (Ugo-Basile, Varese, Italy) was used. Rats were restrained lightly with a towel and the right hindpaw was placed on a small pedestal. A conical, blunt-tipped probe was lowered onto the dorsal paw by depressing a foot pedal; the probe exerted pressure that increased at a constant rate starting at 30 g and increasing 40 g/s to a maximum of 1030 g (25 s). Horizontal locomotor activity was measured using a 20×40×23-cm clear Plexiglas rat cage placed within a photobeam apparatus: 15 photobeams crossed the width of the cage, 2.5 cm apart and 8 cm above the cage floor. Catalepsy was assessed using a bar test (ring stand with a 1.5-cm diameter horizontal bar).

2.3. Surgery

Before gonadectomy, rats were anesthetized with Equithesin (active ingredients: 28.2 mg/kg pentobarbital sodium and 123.3 mg/kg chloral hydrate) i.p. Ovariectomy in females and orchidectomy in males were conducted as described previously (e.g., Stoffel et al., 2003). Immediately after gonadectomy, constant-release Silastic® capsules (0.062 in. i.d./0.125 in. o.d.) were implanted s.c. between the shoulder blades as follows: in females, one 1-mm estradiol-filled or blank capsule; in males, one 10-mm testosterone-filled capsule/100 g body weight, or two 10-mm blank capsules. We have shown previously that in terms of reproductive behavior and physiology, this testosterone treatment regimen yields gonadectomized males that are very similar to gonadally intact males, and this estradiol treatment yields females that are very similar to gonadally intact females in proestrus to estrus (Stoffel et al., 2003). Rats recovered for 21–22 days before behavioral testing.

2.4. Behavioral procedure

All gonadectomized rats were pre-tested twice in the tail withdrawal and paw pressure tests the day before drug testing to establish a baseline value for antinociception and to habituate rats to handling. For gonadally intact rats, baseline testing was conducted on the day of drug testing, so that females would be in same stage during baseline and drug testing. On the drug test day, rats were injected with vehicle or THC i.p. and tested for antinociception on the tail withdrawal and paw pressure tests at 15, 30, 60, 90, 120 and 240 min post-injection (some rats injected with 10 mg/kg THC were also tested at 300, 360 and 420 min post-injection, as paw pressure antinociception was still near-maximal at 240 min). Females were tested only with 5 mg/kg THC, as higher doses produce ceiling effects on some tests such as the paw pressure test (e.g., Tseng and Craft, 2001), such that estradiol-induced enhancement of THC's effects would be hard to discern. For the tail withdrawal test, the rat was wrapped in a soft cloth with the tail hanging freely; the distal 5 cm of the tail was immersed in the water bath, and latency to withdraw the tail from the water was recorded to the nearest 0.01 s with a hand-held stopwatch. If no attempt was made to withdraw the tail within 25 s, the test was terminated. For the paw pressure test, latency to withdraw or attempt to withdraw the paw (jerking the paw away from the probe) was recorded to the nearest 0.5 s. If no attempt was made to escape when 25 s (1030 g) was reached, the paw was removed to avoid tissue damage. Following the paw pressure test at 30, 60 and 120 and 240 min post-injection, rats were placed into locomotor chambers and the number of photobeam breaks in 5 min was recorded. For the catalepsy test, the forepaws were placed on a raised bar and latency to remove both forepaws or climb onto the bar was recorded to the nearest 0.5 s. Rats were taken off the bar after 60 s if no response was made. Catalepsy was assessed only at the 60-min time point, immediately after the paw pressure test. Previously we found that rats cannot be tested repeatedly on the catalepsy bar, as baselines decrease significantly with repeated testing (Tseng and Craft, 2001).

2.5. Determination of estrous cyclicity

Stage of estrous cycle was determined cytologically after vaginal lavage, conducted immediately after testing in all gonadectomized females. Proestrus was identified by the predominance (~80% or more of all cells in sample) of nucleated epithelial cells, estrus was identified by the presence of dense sheets of cornified epithelial cells, diestrus-1 was identified by the presence of scattered, nucleated or cornified epithelial cells and leukocytes, and diestrus-2 was identified by a relative lack of any cells (Freeman, 1988). In gonadally intact females, vaginal smears generally were taken daily for at least 1 week before testing. Females that were determined to be in vaginal estrus or diestrus-1 at approximately 0900 h on the 7th day or later were chosen for testing. No later than 1000 h, the selected rats were injected with vehicle or 5 mg/kg THC, the behavioral tests were conducted, and then a second smear was taken immediately after testing to confirm that the rat remained in the same stage. It should be noted that because females were sampled randomly rather than restricted to 4-day (i.e., short) cyclers, the designation of diestrus-1 is based on vaginal cytology only and does not always reflect the day immediately following the estrous day.

2.6. Drugs

THC was obtained from the National Institute on Drug Abuse (Bethesda, MD), and was prepared in a 1:1:18 cremophor:ethanol:saline solution. THC was prepared in 10 mg/ml concentrations and kept frozen until used; it was administered i.p. in

volumes of 0.5-1 ml/kg. A 1:1:18 cremophor:ethanol:saline solution was used as the vehicle.

2.7. Data analysis

Baseline latencies on the tail withdrawal and paw pressure tests were the mean of two trials conducted for each rat. Latency to respond on the tail withdrawal and paw pressure tests was converted to % maximum possible effect (%MPE): (drug latency − mean baseline latency)/(cutoff latency − mean baseline latency) × 100. For experiments conducted in gonadectomized rats, %MPE antinociception and locomotor (# photobeam breaks) data were analyzed via three-way ANOVA (hormone state (2 levels), THC dose (3 levels), time (4–6 levels, repeated)). To simplify graphic presentation, locomotor activity data (# photobeam breaks) were converted to % control as follows: (# photobeam breaks in THC-treated rats/mean # photobeam breaks in vehicle-treated group at same time point) × 100; these data were then analyzed via ANOVA. Catalepsy data were analyzed via two-way ANOVA (hormone state, THC dose). For the experiment conducted in gonadally intact females, data from females that did not remain in the same stage from before to after testing were not included in analyses ($N=2$). To determine if there were differences in nociceptive or motoric responses after THC treatment among females in estrus vs. diestrus, antinociception and locomotor data were analyzed via three-way ANOVA (estrous stage (2 levels), THC dose (2 levels), time (4–6 levels, repeated). Catalepsy data were analyzed via two-way ANOVA (estrous stage, THC dose). Post-hoc comparisons were conducted using independent *t*-tests with a Bonferroni correction of alpha. Significance level was $P \leq 0.05$ for all statistical tests.

3. Results

3.1. Testosterone modulation of THC's behavioral effects in males

Fig. 1 shows that in gonadectomized males, THC produced dose-dependent antinociceptive effects on the tail withdrawal test ($F(2,42)=19.45$, $P<0.001$), dose- and time-dependent antinociceptive effects on the paw pressure test ($F(10,210)=5.03$, $P<0.001$), dose- and time-dependent suppression of locomotor activity ($F(6,126)=8.42$, $P<0.001$), and dose-dependent catalepsy ($F(2,42)=7.30$, $P<0.002$). There were no significant differences in THC-induced antinociception between gonadectomized males receiving testosterone vs. those receiving no hormone replacement (Fig. 1, top panels). On the paw pressure test (top right panel), the rats injected with 10 mg/kg THC were tested out to 7 h post-injection, by which time antinociception had fallen to approximately 10% MPE in both groups; there were still no differences between no-hormone- and testosterone-treated groups at these later time points (data not shown). In contrast, the locomotor-suppressant effects of

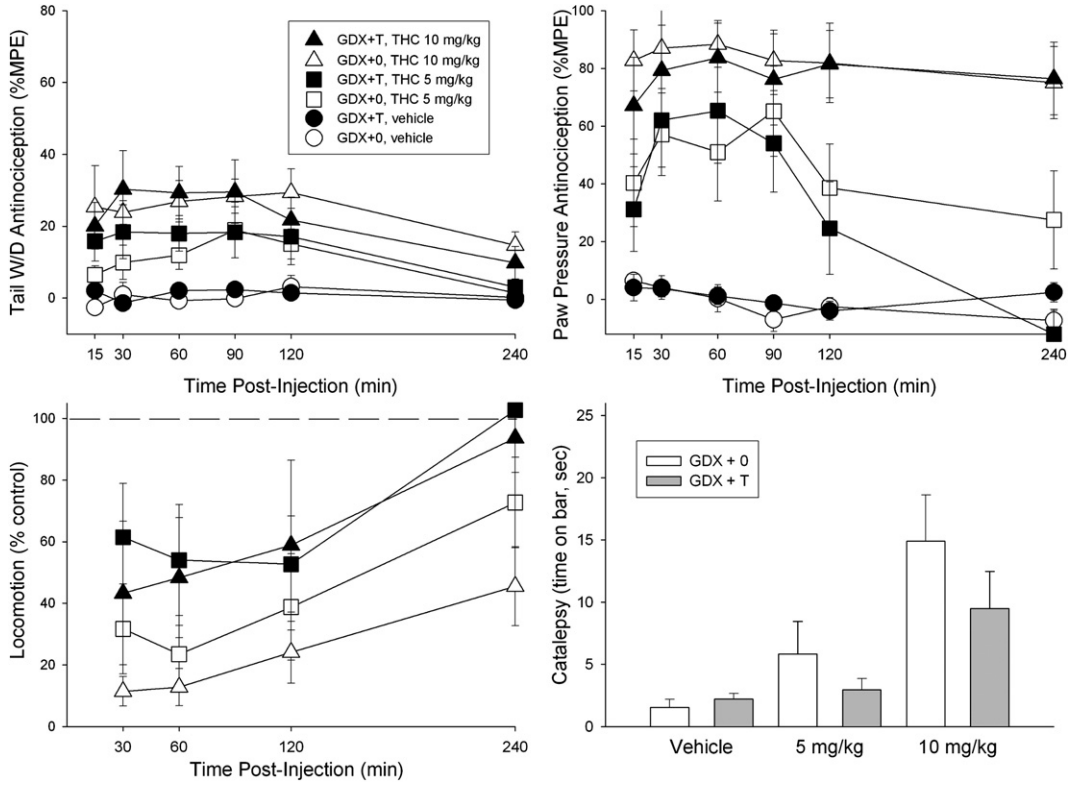

Fig. 1. Time course of THC's effects on the warm water tail withdrawal test (top left panel), the paw pressure test (top right panel), locomotor activity (bottom left panel) and catalepsy (bottom right panel) tests in gonadectomized (GDX) MALE rats implanted with capsules filled with testosterone (T) or nothing (0). Each point is the mean ± S.E.M. of 8 rats.

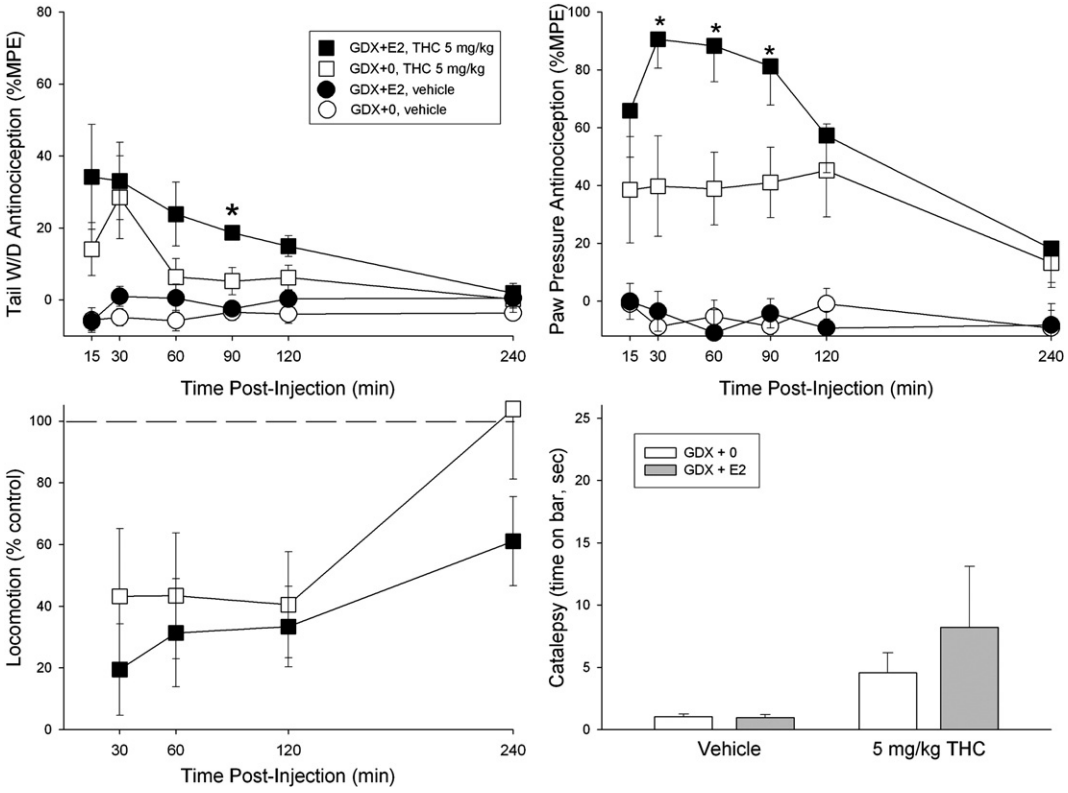

Fig. 2. Time course of the behavioral effects of 5 mg/kg THC in gonadectomized (GDX) FEMALE rats implanted with capsules filled with estradiol (E2) or nothing (0). Other details as in Fig. 1. Each point is the mean±S.E.M. of 7–8 rats. *significantly different than GDX+0 rats, $P \leq 0.05$.

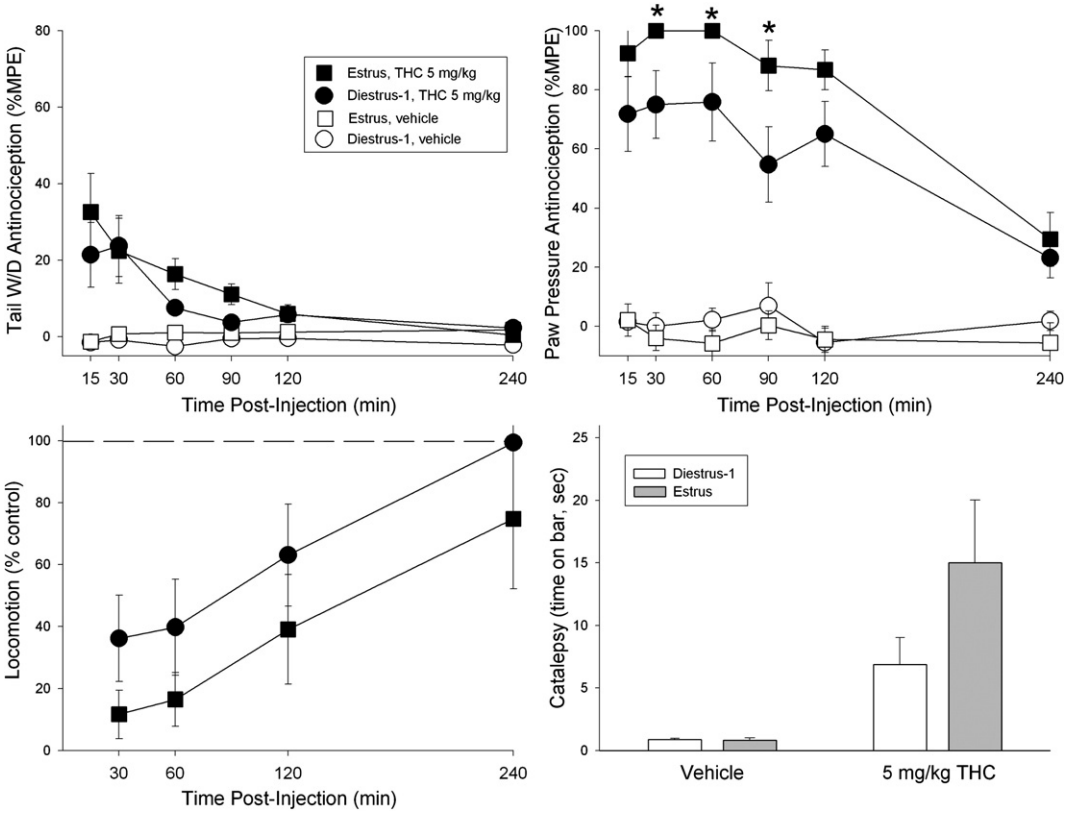

Fig. 3. Time course of the behavioral effects of 5 mg/kg THC in gonadally intact, cycling female rats tested during vaginal estrus or diestrus-1. Other details as in Fig. 1. Each point is the mean±S.E.M. of 8–11 rats. *significantly different than diestrous rats, $P \leq 0.05$.

THC were decreased in testosterone-treated males compared to no-hormone controls (Fig. 1, bottom left panel) ($F(1,28)=5.77$, $P=0.023$). There was no significant difference in raw locomotor scores between gonadectomized males with and without testosterone replacement that received vehicle injections (data not shown). THC's cataleptic effect also appeared to be dampened in testosterone-treated males compared to controls (Fig. 1, bottom right panel), but these group differences were not statistically significant.

3.2. Hormone modulation of THC's behavioral effects in females

Fig. 2 shows that in gonadectomized females, 5 mg/kg THC produced time-dependent antinociceptive effects on the tail withdrawal ($F(5,130)=6.12$, $P<0.001$) and paw pressure tests ($F(5,130)=5.24$, $P<0.001$), time-dependent suppression of locomotor activity ($F(3,78)=6.20$, $P=0.001$), and catalepsy ($F(1,26)=4.33$, $P=0.048$). Estradiol significantly enhanced the antinociceptive effect of THC on the tail withdrawal test (top left panel) ($F(1,26)=3.92$, $P=0.05$) and paw pressure test (top right panel) ($F(1,26)=4.42$, $P=0.05$). In contrast, estradiol did not significantly alter locomotor-suppressing and catalepsy effects of THC (bottom panels). There was no significant difference in raw locomotor scores between females treated with estradiol vs. no hormone that received vehicle injections (data not shown). Fig. 3 shows the effects of 5 mg/kg THC in gonadally intact, cycling females tested during estrus or diestrus-1. Tail withdrawal antinociception was very similar in estrous and diestrous females (top left panel), whereas paw pressure antinociception was significantly greater in estrous than in diestrous females (top right panel) (THC × estrous stage: $F(1,36)=6.28$, $P=0.017$). THC-induced locomotor suppression and catalepsy, although also somewhat greater in estrous than in diestrous females, were not statistically different (Locomotor Activity: $F(1,22)=2.26$, $P=0.15$; Catalepsy: $F(1,38)=2.02$, $P=0.16$). Among vehicle-treated females, estrous females were more active than diestrous females at 30 min post-injection (Estrous Stage × Time: $F(3,48)=2.99$, $P=0.04$) (data not shown).

4. Discussion

We reported previously that THC was more potent and in one case more efficacious in gonadally intact female compared to male rats in inducing antinociception, locomotor suppression and catalepsy (Tseng and Craft, 2001). Other laboratories have reported similar sex differences in behavioral effects of cannabinoids (for review, see Craft, 2005). The present study indicates that these sex differences can be attributed to activational effects of testosterone in males and/or estradiol in females. Specifically, the antinociceptive effects of THC were significantly enhanced by estradiol in females, and the locomotor-suppressant effects of THC were significantly attenuated by testosterone in males. The physiological relevance of estradiol enhancement of THC's paw pressure effect in gonadectomized females was supported by significantly greater paw pressure antinociception in gonadally intact females tested in estrus vs. diestrus. That is, intact females that were tested approximately 24 h after a "high-estradiol state" (when they were in vaginal estrus) showed significantly greater paw pressure antinociception than females tested during a "low-estradiol state" (when they were in vaginal diestrus).

There are several possible mechanisms underlying gonadal hormone modulation of cannabinoid effects in adult rats. First, we have shown previously that sex differences in behavioral effects of THC may be due to sex differences in pharmacokinetic factors: after a systemic injection of THC, female rats had significantly more THC metabolites in brain than males did, and sex differences in antinociceptive effects of THC were abolished by pretreatment with a metabolic inhibitor, SKF525A (Tseng et al., 2004). Liver metabolism of THC differs in female vs. male rats (Narimatsu et al., 1990, 1991, 1992), and estradiol increases 21-hydroxylase activity in hepatocytes from male rats (Endoh et al., 1995). Thus, estradiol enhancement of THC's behavioral effects in female rats may be due to estradiol enhancement of hydroxylation of THC to its active (and more potent) metabolite, 11-OH-THC.

Other possible explanations for hormonal modulation of THC's behavioral effects are based on hormone modulation of cannabinoid pharmacodynamics. For example, chronic estradiol administration to gonadectomized female rats increased cannabinoid receptor density in limbic forebrain whereas chronic testosterone administration to gonadectomized males decreased it (Rodriguez de Fonseca et al., 1993). Estrous cycle-related changes in endocannabinoids such as anandamide and 2-arachidonoyl glycerol have also been observed in some brain areas (Bradshaw et al., 2006). These hormone effects were not observed in all brain areas examined, however, suggesting that hormone modulation of cannabinoid pharmacodynamics is site-specific. Several other studies demonstrate sex differences in, and/or gonadal hormone modulation of cannabinoid receptors, cannabinoid receptor mRNA or cannabimimetic lipid mediators in brain (Gonzalez et al., 2000; Bradshaw et al., 2006) as well as in peripheral tissue (Peroni et al., 2004; Busch et al., 2006), indicating that the cannabinoid system is highly sexually differentiated. To the extent that the different behaviors examined in the present study engage different brain areas may explain why, for example, testosterone modulated motoric but not antinociceptive effects of THC. Motoric effects of THC are known to be mediated by the basal ganglia (e.g., Sañudo-Peña and Walker, 1998), and antinociceptive effects are mediated by multiple areas of the central nervous system, including the amygdala, thalamus, periaqueductal gray-rostral ventromedial medulla, and spinal cord (Lichtman et al., 1996; Meng et al., 1998; Martin et al., 1999; Walker et al., 1999). Many of these areas also contain androgen and/or estrogen receptors (Gréco et al., 1996; Shugrue et al., 1997; VanderHorst et al., 1997; Murphy et al., 1999; Hamson et al., 2004). Site-specific injection of THC and/or hormone will be useful for localizing hormone-cannabinoid interactions in future studies.

In summary, sex differences in behavioral effects of THC in the adult rat may be attributed to activational effects of testosterone in males and estradiol in females, depending on the behavioral endpoint examined.

Acknowledgements

The authors thank Latisha Sternod for excellent technical assistance, and Dr. Michael Morgan for loan of the paw pressure apparatus.

References

Becker, J.B., Arnold, A.P., Berkley, K.J., Blaustein, J.D., Eckel, L.A., Hampson, E., Herman, J.P., Marts, S., Sadee, W., Steiner, M., Taylor, J., Young, E., 2005. Strategies and methods for research on sex differences in brain and behavior. Endocrinology 146, 1650–1673.

Bradshaw, H.B., Rimmerman, N., Krey, J.F., Walker, J.M., 2006. Sex and hormonal cycle differences in rat brain levels of pain-related cannabimimetic lipid mediators. Am. J. Physiol., Regul. Integr. Comp. Physiol. 291, R349–R358.

Busch, L., Sterin-Borda, L., Borda, E., 2006. Effects of castration on cannabinoid CB_1 receptor expression and on the biological actions of cannabinoid in the parotid gland. Clin. Exp. Pharmacol. Physiol. 33, 258–263.

Craft, R.M., 2005. Sex differences in behavioral effects of cannabinoids. Life Sci. 77, 2471–2477.

Endoh, A., Natsume, H., Igarashi, Y., 1995. Dual regulation of 21-hydroxylase activity by sex steroid hormones in rat hepatocytes. J. Steroid Biochem. Mol. Biol. 54, 163–165.

Freeman, M.E., 1988. The ovarian cycle of the rat. In: Knobil, E., Neill, J. (Eds.), The Physiology of Reproduction. Raven Press, New York, NY, pp. 1893–1928.

Gonzalez, S., Bisogno, T., Wenger, T., Manzanares, J., Milone, A., Berrendero, F., Di Marzo, V., Ramos, J.A., Fernadez-Ruiz, J.J., 2000. Sex steroid influence on cannabinoid CB1 receptor mRNA and endocannabinoid levels in the anterior pituitary gland. Biochem. Biophys. Res. Commun. 270, 260–266.

Gréco, B., Edwards, D.A., Michael, R.P., Clancy, A.N., 1996. Androgen receptor immunoreactivity and mating-induced Fos expression in forebrain and midbrain structures in the male rat. Neuroscience 75, 161–171.

Hamson, D.K., Jones, B.A., Watson, N.V., 2004. Distribution of androgen receptor immunoreactivity in the brainstem of male rats. Neuroscience 127, 797–803.

Lichtman, A.H., Cook, S.A., Martin, B.R., 1996. Investigation of brain sites mediating cannbinoid-induced antinociception in rats: evidence supporting periaqueductal gray involvement. J. Pharmacol. Exp. Ther. 276, 585–593.

Martin, W.J., Coffin, P.O., Attias, E., Balinsky, M., Tsou, K., Walker, J.M., 1999. Anatomical basis for cannabinoid-induced antinociception as revealed by intracerebral microinjections. Brain Res. 822, 237–242.

Meng, I.D., Manning, B.H., Martin, W.J., Fields, H.L., 1998. An analgesia circuit activated by cannabinoids. Nature 395, 381–383.

Mize, A.L., Alper, R.H., 2000. Acute and long-term effects of 17beta-estradiol on G(i/o) coupled neurotransmitter receptor function in the female rat brain as assessed by agonist-stimulated [35S]GTPgammaS binding. Brain Res. 859, 326–333.

Murphy, A.Z., Shupnick, M.A., Hoffman, G.E., 1999. Androgen and estrogen (α) receptor distribution in the periaqueductal gray of the male rat. Horm. Behav. 36, 98–108.

Narimatsu, S., Watanabe, K., Matsunaga, T., Yamamoto, I., Imaoka, S., Funae, Y., Yoshimura, H., 1990. Cytochrome P-450 isozymes in metabolic activation of Δ^9-tetrahydrocannabinol by rat liver microsomes. Drug Metab. Dispos. 18, 943–948.

Narimatsu, S., Watanabe, K., Yamamoto, I., Yoshimura, H., 1991. Sex difference in the oxidative metabolism of Δ^9-tetrahydocannabinol in the rat. Biochem. Pharmacol. 41, 1187–1194.

Narimatsu, S., Watanabe, K., Matsunaga, T., Yamamoto, I., Imaoka, S., Funae, Y., Yoshimura, H., 1992. Cytochrome P-450 isozymes involved in the oxidative metabolism of delta 9-tetrahydrocannabinol by liver microsomes of adult female rats. Drug Metab. Dispos. 20, 79–83.

Peroni, R.N., Orliac, M.L., Becu-Villalobos, D., Huidobro-Toro, J.P., Adler-Graschinsky, E., Celuch, S.M., 2004. Sex-linked differences in the vasorelaxant effects of anandamide in vascular mesenteric beds: role of oestrogens. Eur. J. Pharmacol. 493, 151–160.

Rodriguez de Fonseca, F., Cebeira, M., Ramos, J.A., Martin, M., Fernandez-Ruiz, J.J., 1993. Cannabinoid receptors in rat brain areas: sexual differences, fluctuations during the estrous cycle and changes after gonadectomy and sex steroid replacement. Life Sci. 54, 159–170.

Sañudo-Peña, M.C., Walker, J.M., 1998. A novel neurotransmitter system involved in the control of motor behavior by the basal ganglia. Ann. N.Y. Acad. Sci. 860, 475–479.

Shugrue, P.J., Lane, M.V., Merchenthaler, I., 1997. Comparative distribution of estrogen receptor-α and $-\beta$ mRNA in the rat central nervous system. J. Comp. Neurol. 388, 507–525.

Stoffel, E.C., Ulibarri, C.M., Craft, R.M., 2003. Gonadal steroid hormone modulation of nociception, morphine antinociception and reproductive indices in male and female rats. Pain 103, 285–302.

Tseng, A.H., Craft, R.M., 2001. Sex differences in antinociceptive and motoric effects of cannabinoids. Eur. J. Pharmacol. 430, 41–47.

Tseng, A.H., Harding, J.W., Craft, R.M., 2004. Pharmacokinetic factors in sex differences in Δ^9-tetrahydrocannabinol-induced behavioral effects in rats. Behav. Brain Res. 154, 77–83.

VanderHorst, V.G.J.M., Meijer, E., Schasfoort, F.C., Van Leeuwen, F.W., Holstege, G., 1997. Estrogen receptor-immunoreactive neurons in the lumbosacral cord projecting to the periaqueductal gray in the ovariectomized female cat. Neurosci. Lett. 236, 25–28.

Walker, J.M., Hohmann, A.G., Martin, W.J., Strangman, N.M., Huang, S.M., Tsou, K., 1999. The neurobiology of cannabinoid analgesia. Life Sci. 65, 665–673.