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The Effects of Prenatally Administered Endogenous Cannabinoid on Rat Offspring

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WENGER, T., G. FRAGKAKIS, P. GIANNIKOU AND N. YIANNIKAKIS. *The effects of prenatally administered endogenous cannabinoid on rat offspring.* PHARMACOL BIOCHEM BEHAV **58**(2) 537–544, 1997.—We reported previously that the main psychoactive component of marihuana, Δ^9 tetrahydrocannabinol (THC), when injected prenatally, temporarily inhibited the developing hypothalamo–pituitary system in rat offspring. In the present study, we investigated the effects of the recently described endogenous ligand for the central cannabinoid receptor, arachidonyl-ethanolamide (anandamide, ANA), on the postnatal development of the hypothalamo–pituitary axis (HPA) when administered during the third week of gestation. Rat pups were killed every fifth day from delivery to the 20th postnatal day; gonads, pituitary, and rest of body were weighed, and samples were collected for analysis of gonadotropin releasing hormone in the hypothalamus and luteinizing hormone, follicle stimulating hormone, prolactin, and growth hormone in the pituitaries and sera. The effects of ANA and THC were compared. Both ANA and THC caused predominantly inhibitory effects on the measured parameters. The inhibition was most pronounced immediately following delivery, whereas at the end of the investigated period (20th postnatal day) no differences were observed. We conclude that endogenous and exogenous cannabinoids have similar but slightly different effects on the developing HPA and that the action is transitory. We postulate that ANA probably acts via central cannabinoid receptors and/or neuroendocrine receptors to function as a neuromodulator. © 1997 Elsevier Science Inc.

ANA Anandamide Prenatal Hypothalamo–pituitary system Rat Offspring

THE isolation of the main psychoactive component of marihuana, Δ^9 tetrahydrocannabinol (THC), by Mechoulam (15) greatly facilitated research on the pharmacology of cannabis. Howlett and coworkers demonstrated that THC was able to bind to a specific G-protein-coupled receptor in the brain (11). The characterisation of the central cannabinoid receptor (CB1 receptor) has recently been accomplished (13). The postulate that cannabinoids act via a central receptor led to the discovery in porcine brain of the CB1 receptor agonist, an endogenous cannabinoid, arachidonyl-ethanolamide (anandamide, ANA) (5). This compound inhibits the electrically evoked twitch response of the mouse isolated vas deferens at very low concentrations (20), indicating that it possesses pharmacological activity similar to exogenous cannabinoids. Experimental data have also shown that an acute dose of ANA produced antinociception, hypothermia, and hypomotility (19).

Some brain areas are known to contain fewer cannabinoid binding sites than others (25), although the effects produced by THC (and other cannabinoids) in areas such as the hypothalamus (hypothermia), spinal cord (antinociception), and brain stem (suppressing nausea) may well be CB1 receptor mediated (10). These effects may be similar to the receptor-mediated effects of cannabinoid on brain centers involved in the regulation of reproduction.

The effects of THC and/or marihuana on reproduction have been investigated extensively [see references in (28)], but only few studies have dealt with the effects of cannabinoids on pregnancy (28). However, experiments with laboratory animals and retrospective investigations of women who smoked marihuana during pregnancy showed that use of this drug increases the incidence of stillbirths (in animals) and abortion (22,23).

The developing fetal neuroendocrine system depends mainly on the mother's endocrine regulation (17). We demonstrated that THC has an inhibitory action on pituitary gonadotropic function in pregnant rats if administered during the third week of gestation, and that it affects, temporarily, the

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TABLE 1
EFFECTS OF ANA AND THC ON LITTER TRAITS

	Control	ANA	THC
Number of deliveries	16	19	17
Length of gestation (days; mean ± SE)	22.02 ± 0.07	23.12 ± 0.18	22.74 ± 0.13
Average litter size	11.60	12.25	12.05
Number of stillbirths/litter	0.05	3.26	2.53
Male:female ratio of living pups	1.42	0.63	0.93

neuroendocrine system of offspring (26). It was also shown that the hormonal milieu plays an important role in protecting hypothalamic nuclei from cannabinoid exposure during development (8).

The mouse embryo has been found to be a target for cannabinoid ligands, and consequently the effects of cannabinoids during pregnancy could be mediated via cannabinoid receptors (16). In addition, some authors have reported that cannabinoid receptor is present in the early postnatal period and increases in parallel with cerebral proliferation and organization (18). In the present study, using prenatal administration, we compared the effects of THC to those of ANA in rat offspring, because it has been suggested that ANA acts via G-protein-coupled cannabinoid receptors in the brain, with properties similar to those of exogenous cannabinoids (11).

METHODS

Animals

Virgin Sprague–Dawley rats weighing 220–250 g, purchased from Charles River (Hannover, Germany), were paired with males of the same strain in our animal house. The day of finding spermatozoa in the vaginal smears was designated as day 1 of gestation. The animals were maintained under controlled conditions of temperature (20 ± 2°C) and light (lights on 0500 h, off 1900 h) and were provided with water and food (rat pellets) ad lib. All experimental procedures conformed to the *NIH Guide for the Care and Use of Laboratory Animals* (NIH revised version, 1985).

Drug Administration

Both ANA and THC were injected intraperitoneally at a dose of 0.02 mg/kg/day in 0.5 ml vehicle daily between 0930 and 1000 h during the third week of gestation. The injections were made by the same laboratory technician to avoid any unwanted route (e.g., uterine injury) of administration into the mid-lateral part of the abdominal cavity. In our previous study, we demonstrated that THC affects pregnancy in rats only when administered during the third week (26), thus in the present study we injected the drugs between day 14 of pregnancy and the day of delivery. The ethanol solution of THC (of greater than 96% purity) was a generous gift of UN Narcotics Laboratory (Vienna, Austria). ANA [batch #0573; see (1) for procedure] was provided by Professor B. M. Martin (Department of Pharmacology, Virginia Commonwealth University, Richmond, VA, USA). Both drugs were dissolved in a vehicle of propylene glycol and saline solution (1:1). The controls received the vehicle only.

Sample Collection

The offspring of 16 controls and 17 THC- and 19 ANA-treated dams were followed. Each day the male and female offspring of at least two or three mothers were grouped randomly to have 9 or 10 animals of the same sex per group. Sampling was conducted in both sexes every fifth day from the day of delivery (D0) to the 20th postnatal day (D20). After weighing, the animals were killed (9–12/day) by rapid decapitation, always between 0930 and 1030 h. The hypothalami, pituitaries, and gonads were quickly dissected out, weighed, frozen immediately, and then stored at −70°C while trunk blood was collected. Sera were stored at −20°C until subsequent hormonal assays for gonadotropin releasing hormone (GnRH) (hypothalami) and for luteinizing hormone (LH), follicle

TABLE 2
EFFECTS OF PRENATALLY ADMINISTERED ANA AND THC ON BODY WEIGHT OF OFFSPRING

	Days of Age				
	0	5	10	15	20
ANA					
Male	5.54 ± 0.02*	13.27 ± 0.65	21.09 ± 0.67	29.02 ± 1.26	44.12 ± 1.93
Female	5.6 ± 0.19 (12)	12.16 ± 0.57	19.98 ± 0.41	23.35 ± 0.58 (12)	39.98 ± 2.62 (11)
THC					
Male	6.63 ± 0.06** (11)	11.92 ± 0.21**	21.57 ± 0.41	28.11 ± 0.82	38.84 ± 0.79
Female	5.77 ± 0.19	11.86 ± 0.65	20.17 ± 0.43 (12)	26.85 ± 0.75	37.57 ± 0.87 (11)
Control					
Male	6.9 ± 0.06 (12)	12.87 ± 0.35 (11)	19.70 ± 0.87	29.28 ± 1.53	42.62 ± 1.62
Female	5.73 ± 0.19	12.45 ± 0.43	20.27 ± 0.55	25.59 ± 1.00	39.33 ± 1.66

Drugs were administered to dams at 0.02 mg/kg/day over the third week of gestation. Values reported are body weight in g ± SE; sample sizes were 10 offspring/group unless noted otherwise in parentheses.
*$p < 0.002$ and **$p < 0.05$ vs. control (Student's *t*-test).

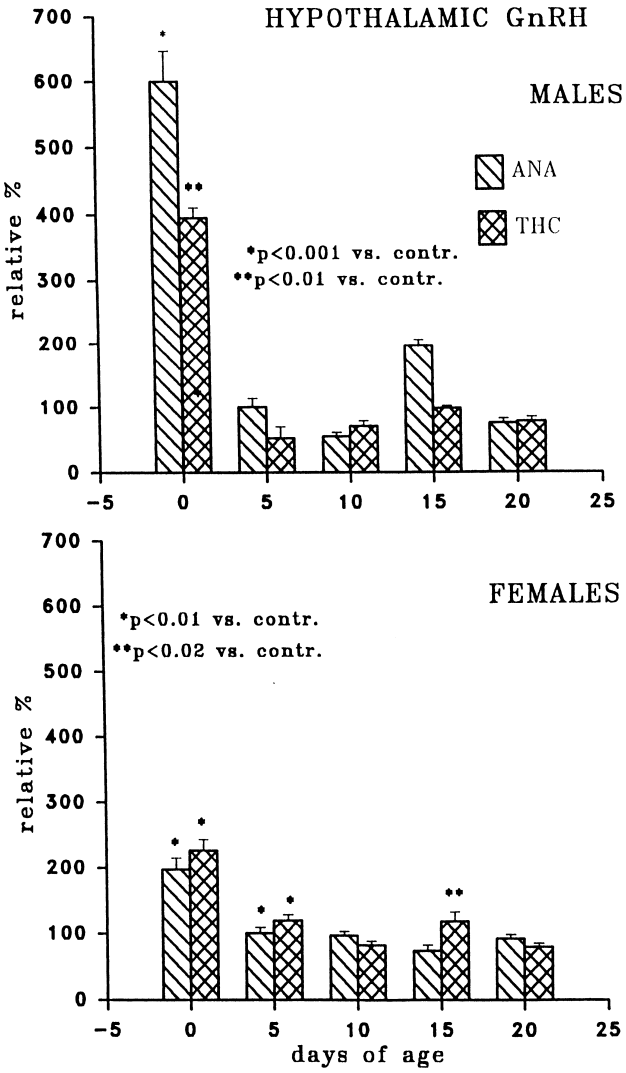
TABLE 3

EFFECTS OF PRENATALLY ADMINISTERED ANA AND THC ON PITUITARY WEIGHT OF OFFSPRING

	Days of Age				
	0	5	10	15	20
ANA					
Male	0.17 ± 0.01	0.54 ± 0.02	1.40 ± 0.05	2.04 ± 0.05	2.00 ± 0.05
Female	0.18 ± 0.04	0.54 ± 0.02	1.55 ± 0.05	2.13 ± 0.05	2.04 ± 0.05
THC					
Male	0.16 ± 0.01	0.51 ± 0.02	1.47 ± 0.06	2.19 ± 0.04	2.11 ± 0.05
Female	0.14 ± 0.01	0.48 ± 0.03	1.62 ± 0.07	2.28 ± 0.08	2.11 ± 0.04
Control					
Male	0.23 ± 0.03*	0.74 ± 0.04*	1.48 ± 0.04	2.01 ± 0.04	2.18 ± 0.01
Female	0.20 ± 0.01**	0.69 ± 0.03*	1.57 ± 0.05	2.08 ± 0.03	2.25 ± 0.10

Drugs were administered to dams at 0.02 mg/kg/day over the third week of gestation. Values reported are weights in mg/pituitary ± SE; sample sizes were 10 offspring/group.

*$p < 0.02$ vs. ANA and THC; **$p < 0.01$ vs. THC (Student's *t*-test).



stimulating hormone (FSH), prolactin (PRL), and growth hormone (GH) (sera and pituitaries). Individual sera were not pooled.

Hypothalamic GnRH was measured according to methods described previously (29). Pituitary hormones were assayed in duplicate at two dilutions using RIA kits supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, Rockville, MD, USA). The assays were carried out according to standard procedures provided with each kit: NIH-RP-2 (LH, sensitivity 440 pg/tube), NIH-RP-1 (FSH, sensitivity 1.8 ng/tube), NIH-RP-3 (PRL, sensitivity 25 pg/tube), and NIH-RP-2 (LH, sensitivity 440 pg/tube; GH, sensitivity 25 pg/tube.

Statistical Analysis

Statistical significance was evaluated by using analysis of variance (ANOVA; Bonferroni multiple comparisons test) and Student's *t*-test; $p < 0.05$ or lower was considered as significant in both analyses.

RESULTS

During the experimental period, no obvious change in the behaviour of the pregnant rats was observed. Both ANA and THC prolonged the gestation period and increased the frequency of stillbirths (Table 1). There was no significant difference in litter size between control and treated animals. No teratological effects in pups were observed. About 10–12% of

FIG. 1. Effects of intrauterine administration of 0.02 mg/kg ANA and THC on hypothalamic gonadotropin releasing hormone content (ng/hypothalamus) in offspring. The bars represent the relative changes in % as compared with the control levels (taken as 100%). The absolute values of the controls (mean ± SE in ng/hypothalamus) were 8.17 ± 2.23 (D0), 49.33 ± 8.34 (D5), 123.11 ± 17.57 (D15), and 101.39 ± 10.24 (D20) in males and 20.13 ± 3.56 (D0), 48.11 ± 8.31 (D5), 72.1 ± 7.3 (D10), 126.35 ± 11.97 (D15), and 265.85 ± 18.91 (D20) in females. Differences were calculated on the basis of the absolute (RIA) values, and statistical analysis was by ANOVA. The overall *F*-value was 27.70 (141 df) in males and 30.183 (143 df) in females.

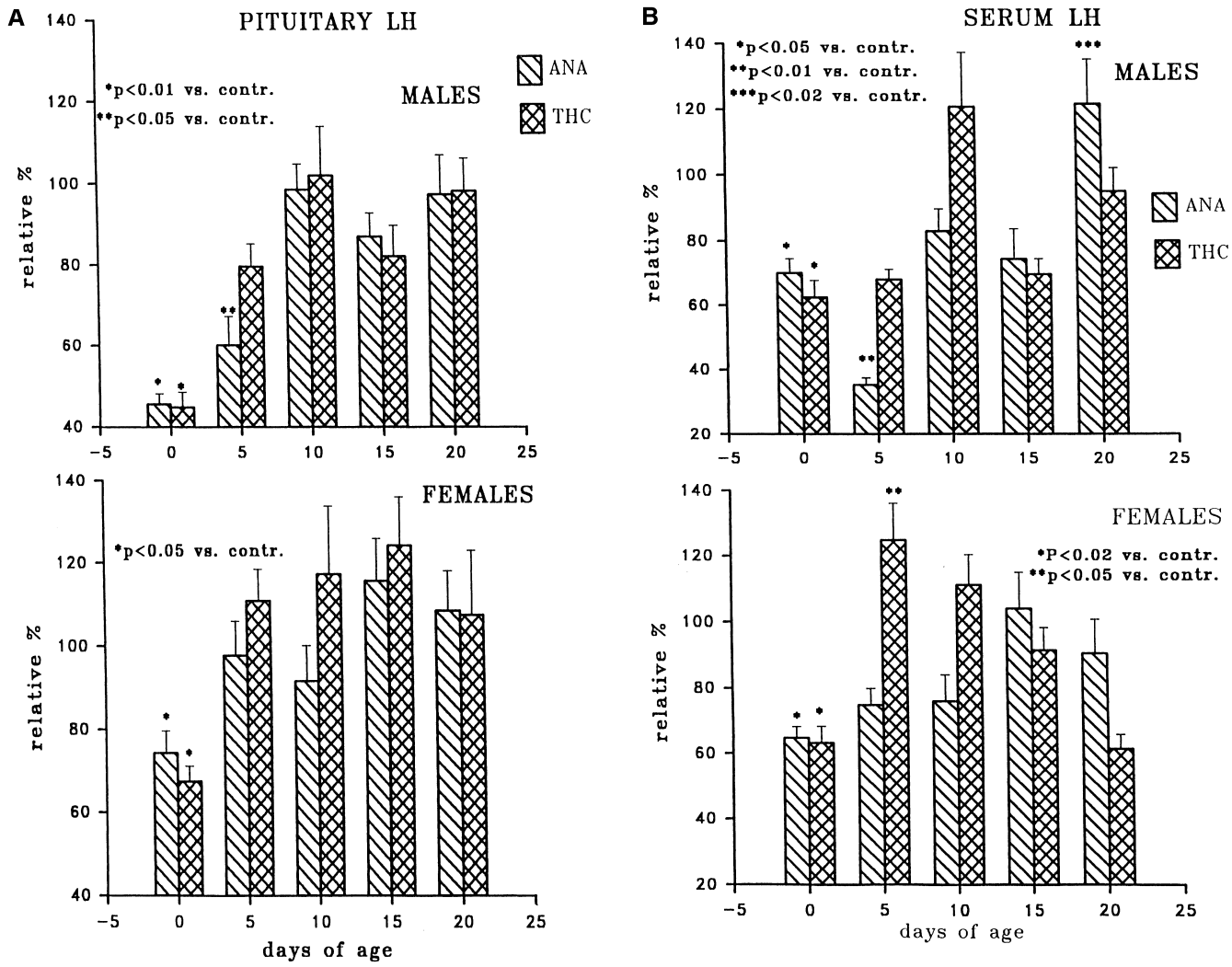


FIG. 2. Effects of intrauterine administration of 0.02 mg/kg ANA and THC on pituitary luteinizing hormone content (μg/pituitary) (A) and on serum luteinizing hormone level (ng/ml) (B) in offspring. The bars represent the relative changes in % as compared with the control levels (taken as 100%). The absolute values of the controls in panel A (mean ± SE in μg/pituitary) were 2.11 ± 0.36 (D0), 9.498 ± 1.78 (D5), 47.611 ± 5.24 (D10), 124.758 ± 15.26 (D15), and 168.438 ± 23.94 (D20) in males and 1.962 ± 0.17 (D0), 16.108 ± 2.04 (D5), 86.133 ± 15.67 (D10), 178.024 ± 11.2 (D15), and 275.239 ± 20.1 (D20) in females, respectively. The absolute values of the controls in panel B (mean ± SE in ng/ml) were 8.64 ± 0.53 (D0), 10.61 ± 0.97 (D5), 8.17 ± 0.87 (D10), 22.63 ± 1.89 (D15), and 14.3 ± 1.57 (D20) in males and 4.87 ± 1.17 (D0), 9.22 ± 1.45 (D5), 7.07 ± 1.26 (D10), 26.12 ± 2.33 (D15), and 7.93 ± 1.11 (D20) in females. Differences were calculated on the basis of the absolute (RIA) values, and statistical analysis was by ANOVA. The overall *F*-value was 15.294 (142 df) in males and 68.131 (141 df) in females in panel A and 12.490 (141 df) in males and 17.533 (143 df) in females in panel B.

the pups were eliminated within the first 12 h after delivery as a result of cannibalism by the mothers. The male/female ratio of living pups changed after the treatments (Table 1): both ANA and THC caused a decrease of the number of surviving female newborns. Table 2 shows the mean weight gain of newborn rats. No differences from controls were detected in female offspring. The males weighed less at delivery (D0) as a result of both ANA and THC treatments, but only THC-treated males showed differences on D5; by D10, no differences in pups' weights were observed. We observed decreased of weight of the pituitaries relative to controls, with a greater effect in THC-treated females (Table 3) on D0 and D5, after which the pituitaries reached a normal weight. ANA caused a significant diminution of testicular weight on D0 ($p < 0.02$). No other differences were detected for gonadal weights at any time in either sex (data not shown).

After either ANA or THC administration, hypothalamic GnRH was higher relative to controls in both sexes on D0. By D5, a more rapid decrease was observed in males. An increase ($p < 0.02$) was present in the GnRH content at D15 in ANA-treated males and THC-treated females (Fig. 1). No marked differences existed in hypothalamic GnRH level at D20. The overall *F*-value for ANOVA was 27.70 in males (141 df) and 30.183 (143 df) in females.

Pituitary LH content was decreased relative to controls after birth in both ANA- and THC-treated animals. At D5, only ANA-exposed males showed lower pituitary LH content. By D10, the pituitary LH content was slightly elevated in THC-

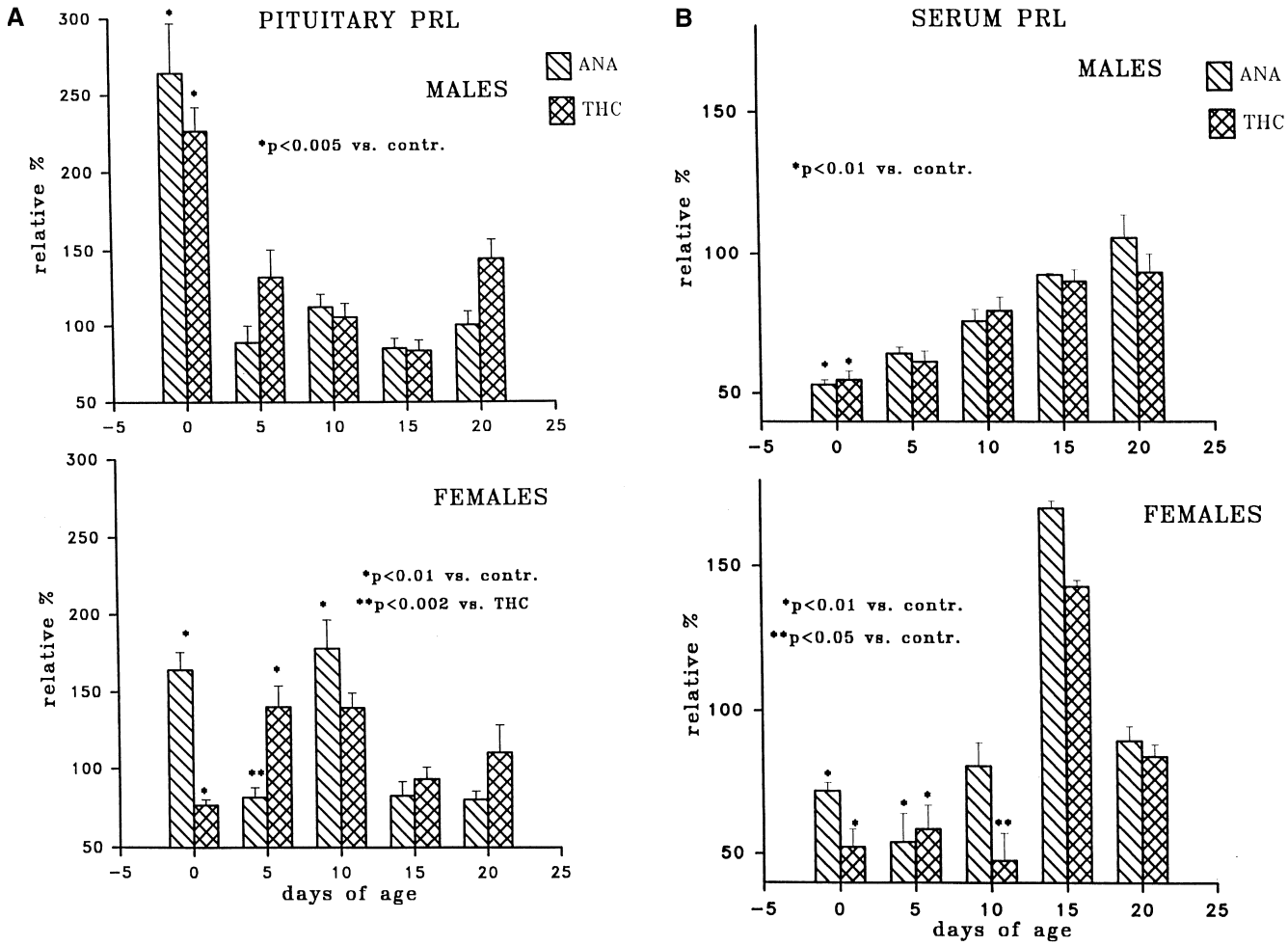


FIG. 3. Effects of intrauterine administration of 0.02 mg/kg ANA and THC on pituitary prolactin content (μg/pituitary) (A) and on serum prolactin level (ng/ml) (B) in offspring. The bars represent the relative changes in % as compared with the control levels (taken as 100%). The absolute values of controls in panel A (mean ± SE in μg/pituitary) were 4.12 ± 0.88 (D0), 63.5 ± 3.48 (D5), 394.94 ± 54.23 (D10), 857.07 ± 55.04 (D15), and 2,426.27 ± 330.01 (D20) in males and 8.36 ± 1.56 (D0), 80.87 ± 7.79 (D5), 301.46 ± 16.18 (D10), 874.5 ± 63.69 (D15), and 3,722.77 ± 356.21 (D20) in females. The absolute values of controls in panel B (mean ± SE in ng/ml) were 3.96 ± 0.23 (D0), 4.18 ± 0.15 (D5), 6.28 ± 0.42 (D10), 9.76 ± 0.68 (D15), and 15.4 ± 2.1 (D20) in males and 4.2 ± 0.11 (D0), 7.3 ± 0.42 (D5), 6.5 ± 1.64 (D10), 3.7 ± 0.92 (D15), and 21.2 ± 2.43 (D20) in females. Differences were calculated on the basis of the absolute (RIA) values, and statistical analysis was by ANOVA. The overall *F*-value was 57.206 (140 df) in males and 69.060 (145 df) in females in panel A and 24.141 (139 df) in males and 27.704 (143 df) in females in panel B.

treated animals as compared with the controls (Fig. 2A). The overall *F*-value for ANOVA of the pituitary LH content was 15.294 (142 df) in males and 68.131 (141 df) in females. ANA and THC had similar effects on serum LH levels in males and females at D0, whereas serum LH was decreased at D5 in ANA-treated males and increased in THC-treated females. The *F*-value for ANOVA was 12.490 (141 df) in males and 17.333 (143 df) in females (Fig. 2B).

Pituitary and serum FSH levels did not change at any time during the experimental period.

Pituitary PRL content in the offspring showed slight differences in the effects of ANA and THC. In males, the pituitary PRL content was elevated after both treatments, whereas in females it was decreased in THC-treated animals on D0 (Fig. 3A). An elevation of PRL level in THC-treated and a decrease in ANA-treated female offspring was observed at D5. At D10, the PRL content was higher in females than in the controls, with a more pronounced increase after ANA treatment. From D15, no changes in pituitary PRL was detected in either sex as compared with the controls (Fig. 3A). The overall *F*-value for ANOVA was 57.206 (140 df) in males and 69.060 (145 df) in females. Serum PRL was diminished in both sexes after both treatments at D0 and up to D10 in females only (Fig. 3B). The *F*-value for ANOVA was 24.141 (139 df) in males and 27.704 (143 df) in females.

GH content in the pituitaries was decreased following both ANA and THC treatment in females and following ANA treatment in males; however, hypophyseal GH levels were significantly different between ANA- and THC-treated males ($p < 0.005$) (Fig. 4A). Lower GH levels at D5 were detected in both sexes after THC treatment. The *F*-value for ANOVA was 47.558 (141 df) in males and 53.368 (136 df) in females. Serum GH levels at D0 and D5 were decreased in both males and females following both treatments. Serum GH was ele-

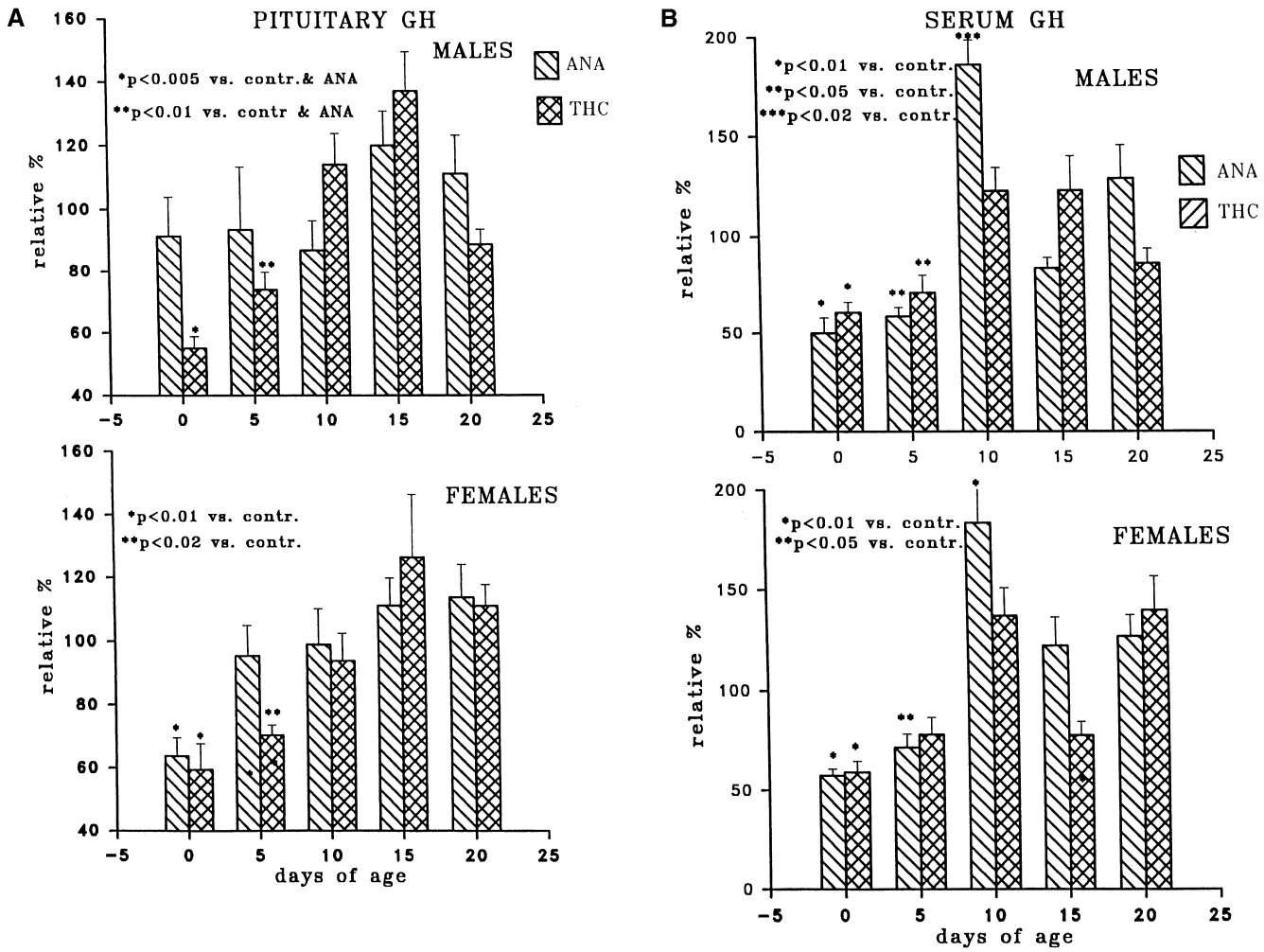


FIG. 4. Effects of intrauterine administration of 0.02 mg/kg ANA and THC on pituitary growth hormone content (μg/pituitary) (A) and on serum growth hormone level (ng/ml) (B) in offspring. The bars represent the relative changes in % as compared with the control levels (taken as 100%). The absolute values of controls in panel A (mean ± SE in μg/pituitary) were 1.48 ± 0.19 (D0), 4.12 ± 0.24 (D5), 12.57 ± 1.54 (D10), 18.17 ± 4.94 (D15), and 36.96 ± 2.89 (D20) in males and 1.62 ± 0.21 (D0), 5.58 ± 0.4 (D5), 11.94 ± 1.43 (D10), 15.52 ± 1.61 (D15), and 29.25 ± 3.50 (D20) in females. The absolute values of controls in panel B (mean ± SE in ng/ml) were 6.94 ± 0.86 (D0), 13.19 ± 2.23 (D5), 11.134 ± 2.09 (D10), 15.33 ± 2.56 (D15), and 11.11 ± 4.23 (D20) in males and 4.70 ± 0.73 (D0), 11.29 ± 1.77 (D5), 10.38 ± 1.55 (D10), 18.06 ± 1.81 (D15), and 7.97 ± 0.87 (D20) in females. Differences were calculated on the basis of the absolute (RIA) values, and statistical analysis was by ANOVA. The overall *F*-value was 47.558 (141 df) in males and 53.368 (136 df) in females in panel A and 3.847 (136 df) in males and 7.644 (140 df) in females in panel B.

vated at D10 in ANA-treated males and females (Fig. 4B). The overall *F*-value for ANOVA was 3.847 (136 df) in males and 7.644 (140 df) in females.

DISCUSSION

To our knowledge, the present results are the first to describe the comparative effects of prenatally administered ANA and THC on the hypothalamo–pituitary axis (HPA) in rat offspring.

The data show that intrauterine ANA administration resulted in a temporary inhibition of the developing HPA. The effects on the HPA produced by ANA were similar to, but not always the same as, the effects produced by THC. ANA has been suggested (30) to parallel THC in activating the HPA via a central mechanism that involves the secretion of corticotropin releasing factor (CRF). Dalterio et al. (3) postulated that prenatal exposure to THC would produce long-term alterations in pituitary–gonadal function. Our previous findings (27) and the present results show that when the dose of cannabinoids is low, the effect is only a temporary inhibition, which is in good accordance with other descriptions of the pharmacological properties of ANA (9).

ANA was proposed to be an endogenous neural messenger (6). The possibility of the existence of an anandaergic neuronal system has also been raised (4). The observed alterations in neuroendocrine parameters may be mediated by changes in biogenic amines, as was postulated elsewhere (8). The differences between the effects of ANA and THC may vary as a function of route of administration, as has been reported with regard to their antinociception properties (24).

Acute administration of THC suppressed secretion of GH

(7). THC can also inhibit the hypothalamo–pituitary control of normal episodic GH secretion in the rat, as described elsewhere (21). Our results further support this action of cannabinoids in the regulation of GH secretion. No changes in FSH levels were observed in the present study. This observation is in accordance with the existence of a postulated FSH releasing factor (16), the release of which is not suppressed by either ANA or THC.

Multiple doses of cannabinoids lead to an increase in the maternal plasma concentration of these drugs or their metabolites. It has been reported that the placenta lacks a barrier to the passage of several drugs, including exogenous cannabinoids [see (28)]. We may suppose that ANA, the endogenous cannabinoid, acts in the same way as THC and crosses the placental barrier if its blood concentration is high enough, because ANA is not degraded while present in the blood circulation (19).

Hutchings et al. (12) found a significant increase of the proportion of male offspring in THC-treated mothers. In our present and previous (26) experiments, the males were more affected by prenatal THC and ANA treatments. Hutchings et al. (12) administered a much higher dose (50 mg/kg) than we used, and the different results might be attributed to the different doses. It seems reasonable to assume that responsiveness of males to cannabinoids may be dose dependent.

The effects of both exogenous and endogenous cannabinoids were transitory. Higher amounts of these drugs (or their metabolites) would be expected in the newborns (D0), and after delivery, when the litters are no longer exposed to cannabinoids, levels in the offspring should decrease. The decrease of the pharmacologically active cannabinoids in the rats may be the most important factor in the temporary nature of the effects.

In summary, we conclude that ANA, similarly to THC, produces mainly inhibitory effects on the HPA. ANA has been suggested to act as a neurotransmitter or neuromodulator (2), perhaps through the central cannabinoid receptor present in the developing central nervous system (14,18). We postulate that ANA acts at hypothalamic levels as a neuromodulator, and this might be the cause of its slightly different actions vs. those of the exogenous cannabinoids. Further studies are needed to elucidate the physiological role of ANA in the regulation of neuroendocrine processes. If our assumption based on the present results in rats is correct, it might also be extrapolated to other species, e.g., humans.

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