Results. In the drug stimulus generalization tests, presented in table 1, we found that the (−)-enantiomer (**II**) was ca 87 times more active than natural delta-1-THC (**I**) in the rat, and ca 73 times more active in the pigeon. The (+)-enantiomer (**III**) was inactive at doses ca 1000 times and ca 4500 times (for rats and pigeons respectively) higher than those of the ED_{50} of the (−)-enantiomer (**II**).

The same type of results were observed in the rotarod test in rats, presented in table 2. The (−)-enantiomer (**II**) was ca 260 times more potent than natural delta-6-THC (**IV**); the (+)-enantiomer (**III**) was inactive at doses ca 2000 times higher than those of the ED_{50} of the (−)-enantiomer (**II**).

Qualitatively the same type of results were obtained in the mouse ring test (see table 3): the (+)-enantiomer (**III**) was inactive in all doses tested; the (−)-enantiomer (**II**) was several hundred times more active than natural delta-6-THC (**IV**).

Discussion. The above results show clearly that in the enantiomeric pair of THC-type compounds (**II**) and (**III**) psychotropic activity resides solely in the (−)-(3R,4R) enantiomer (**II**), the (+)-(3S,4S) being inactive at doses up to several thousand times higher than the enantiomer ED_{50} of the (−)-(3R,4R) enantiomer (**II**). As mentioned above, this is in contrast to results obtained with other THC-type enantiomeric pairs [3]. As we believe that these differences are due to the presence of impurities of (3R,4R) enantiomers in the (3S,4S) enantiomers tested up till now, we are at present looking at synthetic routes leading to these (3S,4S) enantiomers with absolute stereochemical purity. However, on the basis of well-established structure-activity relationships [13] our results are very likely to be of general value. One of the structural changes of the THC molecule introduced by us, namely the hydroxyl group at C-7, is known to retain or increase THC activity. 7-Hydroxy-delta-1-THC and 7-hydroxy-delta-6-THC are major active primary metabolites of delta-1-THC and delta-6-THC, respectively [14]. The second change, the replacement of the n-pentyl side chain with a 1,1-dimethyl heptyl side chain, is also known to cause an increase in biological activity in all THC-type compounds tested so far [13]. We do not expect that these two chemical modifications would cause a pattern of activity different from that observed in other cannabinoids.

The (−)-(3R,4R) enantiomer (**II**) is one of the most psychotropic THC-type compounds in rodents and in pigeons reported so far. The ED_{50} for psychotropic activity of delta-1-THC and delta-6-THC are known for rodents and pigeons as well as for humans [7, 8, 14, 15]. If the animal to human ratios of activity established for these THC's are also valid for compound (**II**), marijuana-type psychotropic effects with (**II**) in man will appear at total doses as low as 0.1–0.2 mg. The psychotropically inactive enantiomer (**III**) is analgetic and antiemetic. These observations will be reported separately.

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2 Presented in part at a meeting at the US National Institute on Drug Abuse, Washington, D.C., October 1986, see NIDA Research Monographs *79* (1987) 15.

3 Dewey, W. L., Martin, B. R., and May, E. L., in: Handbook of Stereoisomers: Drugs in Psychopharmacology, pp. 317–348. Ed. D. F. Smith. CRC Press, Boca Raton, Fla. 1984; Mechoulam, R., Lander, N., Varkony, T. H., Kimmel, I., Becker, O., Ben-Zvi, Z., Edery, H., and Porath, G., J. med. Chem. *23* (1980) 1068.
4 Martin, B. R., Pharmac. Rev. *38* (1986) 45.
5 Mechoulam, R., Braun, P., and Gaoni, Y., J. Am. Chem. Soc. *89* (1967) 4552; *94* (1972) 6159.
6 Hiltunen, A. J., and Järbe, T. U. C., Neuropharmacology *25* (1986) 133.
7 Järbe, T. U. C., Swedberg, M. D. B., and Mechoulam, R., Psychopharmacology *75* (1981) 152.
8 Ferster, C. B., and Skinner, B. F., Schedules of Reinforcement. Appleton-Century-Crofts, New York 1957.
9 Järbe, T. U. C., Hiltunen, A. J., Lander, N., and Mechoulam, R., Pharmac. Biochem. Behav. *25* (1986) 393.
10 Consroe, P., and Wolkin, A., J. Pharmac. exp. Ther. *201* (1977) 26.
11 Consroe, P., Martin, A., and Mechoulam, R., in: Marihuana '84, Proceed. Oxford Symp. Cannabis, pp. 705–712. Ed. D. J. Harvey. IRL Press, Oxford 1985.
12 Pertwee, R. G., Br. J. Pharmac. *46* (1972) 753.
13 Razdan, R. K., Pharmac. Rev. *38* (1986) 75; Mechoulam, R., and Feigenbaum, J. J., Progr. med. Chem. *24* (1987) 159.
14 Harvey, D. J., and Paton, W. D. M., Rev. Biochem. Toxic. *6* (1984) 221.
15 Dewey, W. L., Pharmac. Rev. *38* (1986) 151.

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Hypothalamo-hypophyseal-gonadal function in the rat following administration of the novel and selective D-1 agonist CY 208–243

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Summary. The effects of the novel and selective dopamine D-1 agonist CY 208–243 on the rat hypothalamo-hypophyseal-gonadal (HHG) axis were studied. CY 208–243 did not modify the concentration of luteinizing hormone (LH) in serum from female or male rats, and had no effect upon opiate antagonist-induced stimulation of LH secretion in male rats. CY 208–243 did not inhibit ovulation in cycling female rats. Thus, D-1 receptor activation by systemic drug administration does not alter HHG function in rats.
Key words. Hypothalamo-hypophyseal-gonadal axis; dopamine D-1 neurotransmission.

Dopamine D-1 neurotransmitter systems have been implicated in the central regulation of gonadotrophic hormone secretion in the rat. D-1 receptors have been identified in the median eminence [1], and a correlation between hypothalamic dopamine-stimulated adenylate cyclase activity, which is taken as an index for selective D-1 activation, and gonadotrophin release, has been observed [2]. Recently, a discrete stimulatory effect of a selective D-1 agonist on LH release within the zona incerta (Z.I.) of the hypothalamus has been described [3].

The novel D-1 agonist CY 208–243, a (–)-4,6,6a,7,8,12b-hexahydro-7-methylindolo[4,3-ob]phenanthradine, has recently been developed for use in CNS disorders and has been characterized in detail[4]. This compound exhibits high affinity binding to brain D-1 recognition sites. CY 208–243 stimulates adenylate cyclase activity in rat striatum homogenates, but is inactive in in vitro and in vivo tests which identify actions at dopamine D-2 receptors (Markstein[4], and personal communication).

In the present studies, we have investigated the effects of CY 208–243 on the hypothalamo-hypophyseal-gonadal axis of the rat.

Materials and methods. Three rat models were used in the present studies:

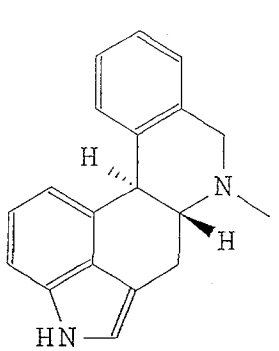
1. Juvenile female rats: The rats, of the Wistar strain, were delivered to our animal quarters at 22–23 days of age. Experiments were performed 2–4 days later. Average body weight at the time of the experiments was 45 g.
2. Adult male rats: 6–8-week-old rats of the Wistar strain were used. They were housed in our animal quarters for at least 3 days prior to an experiment.
3. Adult female rats: These experiments were also performed on Wistar rats. Cycles were determined by daily vaginal smears. Only rats which exhibited regular cyclicity were used. Experiments were performed on animals in the proestrous and diestrous stages.

All animals were housed in group cages prior to the experiments. Ambient temperature in our animal quarters was 22–23 °C, with artificial illumination between 06.00 h and 18.00 h. Food and water were available ad libitum. One day prior to an experiment the animals were placed in individual cages. They were removed to an adjacent room for substance (or vehicle) application. Decapitation was also performed in a separate room.

The chemical structure of the D-1 agonist CY 208–243 is depicted in the figure. The compound was dissolved in water with tartaric acid, and was injected s.c. in the dose range 0.032–3.2 mg/kg. Control animals were given vehicle alone. After death, trunk blood was collected on ice and centrifuged at 4 °C. Sera were stored at – 30 °C for subsequent determinations of luteinizing hormone (LH). LH was measured by specific radioimmunoassay (RIA), using an antiserum produced in house[5].

Results and discussion. Experiment 1. CY 208–243 was injected s.c. to juvenile female rats at 0.32 or 1.0 mg/kg. The animals were sacrificed 30 min or 60 min thereafter. For comparison, naloxone was given at 1.0 mg/kg s.c. CY 208–243 had no effect whatsoever on serum LH concentrations. Naloxone induced the expected rise in circulating LH concentrations (table 1).

Experiment 2. CY 208–243 was administered to adult male rats at 0.032, 0.32 or 3.2 mg/kg, s.c. Decapitation times were at 2 or 4 h. The opiate agonist bremazocine was taken as a reference compound. No effects of CY 208–243 on circulating LH concentrations were seen. Bremazocine, however, suppressed serum LH concentrations 4 h following application, as expected (table 2).



The chemical structure of CY 208–243.

Table 1. Concentration of luteinizing hormone in the serum of juvenile female rats, 30 min or 60 min after administration of CY 208–243 or naloxone

Drug dose (mg/kg s.c.)		Serum LH (ng/ml) 30 min	60 min
Control		34 ± 3	91 ± 5
CY 208–243	0.32	43 ± 4	77 ± 2
	1.0	40 ± 2	88 ± 2
Naloxone	1.0	156 ± 17**	154 ± 30**

The data are expressed as group arithmetic means ± SEM's. ** $p < 0.01$ in comparison with controls (Student's t-test). $N \geq 6$.

Table 2. Concentration of luteinizing hormone in the serum of male rats, 2 or 4 h after application of CY 208–243

Drug dose (mg/kg s.c.)		Serum LH (ng/ml) 2 h	4 h
Control		72 ± 6 (8)	84 ± 10 (10)
CY 208–243	0.032	73 ± 9 (5)	78 ± 4 (6)
	0.32	78 ± 13 (5)	74 ± 7 (6)
	3.2	75 ± 7 (5)	88 ± 2 (6)
Bremazocine	3.2	–	58 ± 1**

Arithmetic means ± SEM's are given. The numbers in parentheses refer to number of animals. ** $p < 0.01$ in comparison with controls (Student's t-test).

Table 3. Concentration of luteinizing hormone in the serum from adult male rats, 2 h after administration of CY 208–243 alone or in combination with the opiate antagonist naltrexone

Naltrexone	Serum LH (ng/ml) 0	10 mg/kg p.o.
Vehicle only	36 ± 4	63 ± 8**
CY 208–243	36 ± 2	67 ± 16**

** $p < 0.01$ in comparison with the Naltrexone 0 dose group (Student's t-test. $N \geq 6$.

Experiment 3. The aim of this experiment was to explore the possibility of an interaction of CY 208–243 with opiate antagonist-induced LH secretion. CY 208–243 was administered to adult male rats s.c. at 0.56 mg/kg, alone or in combination with the opiate antagonist naltrexone, 10 mg/kg orally (p.o.). CY 208–243 alone had no effect on serum LH concentrations, nor did this substance alter the LH secretory response induced by naltrexone at 10 mg/kg (table 3).

Experiment 4. Ovulation inhibition. CY 208–243 was administered at 3.2 mg/kg s.c., at 16.00 h on the afternoon prior to proestrous, and again at 11.00 h on the morning of proestrous. Control animals received vehicle only. CY 208–243 did not inhibit ovulation in any of the rats.

The animal models employed herein represent sensitive and reliable test systems for the identification of pharmacological agents with stimulatory or inhibitory effects on the HHG axis. Marked LH secretion stimulation can easily be evoked in the juvenile female rat by administration of appropriate substances, such as opiate antagonists[6–8]; this has been confirmed in the present experiment with naloxone. Smaller but still consistent increments in circulating LH concentrations can also be produced in the adult male rat[9], again confirmed in the present studies with the opiate antagonist naltrexone. The adult male rat model is also useful for identifying inhibitory influences on LH secretion[9,10] as revealed in these studies by the results with bremazocine. In addition, the ovulation test is a sensitive model for the identification of suppressive effects upon the HHG axis[11,12].

CY 208–243 is centrally active in the dose range used in the present experiments. This is attested to by its effects in rats with unilateral 6-hydroxydopamine-induced lesions of the substantia nigra, and by its induction of sniffing and grooming behaviors[4].

CY 208–243 failed to influence the HHG axis in all of the various experimental models investigated herein. No evidence for either stimulatory or inhibitory effects of the drug on LH secretion or on ovulation was observed. Thus, CY 208–243, in therapeutically and pharmacologically relevant doses, does not affect the HHG axis in rats.

The present data should be considered in the light of previous reports. Local injections of the D-1 antagonist SCH 23390 into the zona incerta (Z.I.) of the hypothalamus suppressed ovulation in cycling female rats; dose-dependence could not, however, be demonstrated. A local stimulatory effect of the D-1 agonist SKF 38393 on LH secretion in estrogen-primed ovariectomized rats was also reported, but the duration of action was very short[3]. Those results were taken as evidence for the existence of a discrete D-1 neurotransmitter system located within the Z.I. which regulates the secretion of LH in female rats. The present study, in which our novel and selective D-1 agonist failed, in systemic doses which have been demonstrated to have clear central effects, to affect the HHG axis in various experimental paradigms, suggests that this discrete D-1 neuronal system is probably not of major importance in the normal function of the HHG axis. Rather, its previously demonstrated involvement in LH secretion regulation is probably minimized by other (e.g. opiate, serotoninergic) transmitter systems, acting in a counter-regulatory fashion.

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1 Fuxe, K., Agnati, L. F., Benfenati, B., Andersson, K., Camurri, M., and Zoli, M., Neurosci. Lett. *43* (1983) 185.
2 Barr, G. A., Ahn, H., and Makman, M. H., Brain Res. *277* (1983) 299.
3 James, M. D., Mac Kenzie, F. J., Tuohy-Jones, A., and Wilson, C. A., Neuroendocrinology *45* (1987) 348.
4 Markstein, R., Seiler, M. P., Vigouret, J. M., Urwyler, S., Enz, A., and Dixon, K., Proceedings VIth int Congr. of Catecholamines (Jerusalem, June 14–19, 1987). Ed. M. Sandler. in press.
5 Anderson, F. B., O'Grady, J. E., and Niederer, W., Biochem. Soc. Transact. *1* (1973) 496.
6 Blank, M. S., Panerai, A. E., and Friesen, H. G., Science *203* (1979) 1129.
7 Schulz, R., Wilhelm, A., Pirke, K. M., and Herz, A., Life Sci. *31* (1982) 2167.
8 Sylvester, P. W., Sarkar, D. K., Briski, K. P., and Meites, J., Neuroendocrinology *40* (1985) 165.
9 Bruni, J. F., Van Vugt, D., Marshall, S., and Meites, J., Life Sci. *21* (1977) 461.
10 Markó, M., and Römer, D., Life Sci. *33* (1983) 233.
11 Markó, M., and Flückiger, E., Neuroendocrinology *30* (1980) 228.
12 Markó, M., and Flückiger, E., Experientia *30* (1974) 1174.

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Release of 3,5,3′-triiodothyronine, thyroxine and thyroglobulin from TSH-stimulated mouse thyroids in the perifusion system

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Summary. We established a perifusion system using mouse thyroid glands. In this system, TSH increased the release of T_3 and T_4 significantly, and the response of thyroglobulin to TSH was delayed in comparison with that of T_3 and T_4.
Key words. Thyroglobulin; TSH; T_3; T_4; mouse; thyroid; perifusion system.

Thyroglobulin (Tg) is synthesized in the follicle epithelial cells of the thyroid gland and secreted into the blood circulation in normal subjects[1]. Although the precise mechanism for the secretion of Tg still remains unknown, TSH is one of the factors which control Tg secretion[1].

Previously, we reported the response of thyroid hormones to TSH in a perifusion system with rat thyroid glands[2,3]. In the present study, the same perifusion and the specific RIA system for mouse Tg were employed in order to clarify Tg release from TSH-stimulated mouse thyroid glands.

Materials and methods. Perifusion systems: Mouse thyroid glands were used in perifusion systems as previously described[2,3]. After ether anesthesia, thyroid glands were removed from male BALB/c mice (8–10 weeks old) and bisected. The lobes obtained were preincubated in Krebs-Ringer bicarbonate buffer containing 0.1% glucose and 0.3% BSA (KRBG) at 37 °C for 90 min, and twenty pieces were placed in a chamber with a capacity of 0.25 ml. The thyroid pieces were then perifused with the same buffer at a flow rate of 1.9 ml/20 min. TSH (10 mU/ml) was added 60 min after the start of perifusion, and the procedure was continued for 3 h. Perifusates were collected at 20-min intervals and stored at – 20 °C until assay of contents.

T_3, T_4 and Tg RIA: We used a previously described RIA method for measurement of T_3 and T_4[2]. Tg concentration in each of the perifusates was measured using a double-antibody RIA method, following the procedure of Kawamura et al.[4]. Mouse Tg was prepared by the method of Tarutani et al.[5]. Antiserum against Tg was obtained from albino rabbits which had been initially injected intracutaneously with 750 µg of mouse Tg in complete Freund's adjuvant, and 28 days later with 500 µg of mouse Tg. Antiserum was used at 20,000-fold dilution. Amounts of T_3 and T_4 up to 5000 ng per tube did not interfere with the binding of ^{125}I Tg. Each assay was done in duplicate.

For the morphological observation, the thyroid tissues after 3-h stimulation were fixed in 10% formaldehyde solution, embedded in paraffin, and processed for light microscopy. The tissues were stained with hematoxylin-eosin.

TSH was obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents used were of analytical grade. The statistical significance of the data obtained was determined by Student's t-test.

Results. Figure 1 shows the profiles of the release of T_3, T_4 and Tg from the mouse thyroid specimens. TSH (10 mU/ml) gradually increased the release of T_3 for 3-h stimulation. T_3