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Influence of 2 Br-α-ergocryptine (CB 154) on the secretion of prolactin, LH, FSH and testosterone and on testicular growth in rams subjected to different photoperiods

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Summary. The influence of 2-Br-α-ergocryptine (CB 154) on the secretion of gonadotrophins and on testicular function has been studied in rams subjected to either a normal photoperiod or an abnormal photoperiod causing hyperprolactinaemia. The CB 154 treatment significantly lowered the mean frequency of LH and testosterone pulses in hyperprolactinaemic animals as compared to solvent-treated ones. Also, only those groups subjected to an abnormal photoperiod (groups 2 and 3) exhibited a significant rise in the frequency of LH and testosterone peaks after CB 154 was withdrawn. During treatment, plasma FSH concentrations increased significantly only in group 1 which was subjected to normal photoperiodic variations. Testicular growth was delayed in CB 154-treated rams compared to solvent-treated ones only in group 3 (hyperprolactinaemic).

Introduction.

Prolactin (Prl) affects the male (Bartke, 1980) as well as the female reproductive function. Prl action has been studied in male rat (Harper *et al.*, 1976), hamster (Bartke, 1976) and lamb (Ravault *et al.*, 1977) but its role in the ram is still unknown. Barenton and Pelletier (1980) showed that during hypo- or hyperprolactinaemia, the number of testicular LH receptors remained constant in rams but that hypoprolactinaemic rams showed a delayed testicular growth. The present paper analyses in the ram the effect of hyper- and hypoprolactinaemia on LH, FSH and testosterone (T) secretion and on testicular growth which is controlled by these three hormones (Courot and Ortavant, 1981). The animals were rendered hyperprolactinaemic by using a light schedule described previously (Ravault and Ortavant, 1977) and hypoprolactinaemic by injecting 2 Br-α-ergocryptine (CB 154).

Material and methods.

Forty adult Prealpes rams, subjected to a natural photoperiod for 3 months (end of September to the beginning of January) were housed 5 to a 10 m^2-pen with a light intensity of about 300 lux at eye-level. They were given an artificial light schedule or subcutaneous injections of CB 154 or both (fig. 1a) and then divided into three groups of 10 rams each, except for group 3 containing 20 rams. Five (or 10) rams were treated with CB 154 and 5 (or 10) with solvent. The first group was submitted to normal photoperiodic variations ; the second group received 8 h of light, and the third group 7 h of light + a light pulse of 1 h situated 16 h after dawn. Blood was sampled from the jugular vein for 25 successive hours on January 4th, February 9th, March 23rd, April 24th and June 12th ; these dates were called sequence 1 (S_1), S_2, S_3, S_4 and S_5, respectively (fig. 1b). During the hours of darkness, the blood was sampled using a green light of very low intensity. In addition, 10 ml of blood were taken once a week in the morning. The testicular diameters were measured every 15 days with a caliper.

The CB 154 treatment began on January 5th and ended on April 25th. Each treated animal was injected twice a day at 8.30 a.m. and 5.00 p.m. with 2 mg of CB 154. This drug was dissolved in a minimal volume of 70 p. 100 (V/V) ethanol and then mixed with water and tartaric acid (same weight as CB 154) to a final concentration of 2 mg/ml.

The rams were fed a mixture of dehydrated maize, oats and lucerne ; water was supplied *ad libitum*.

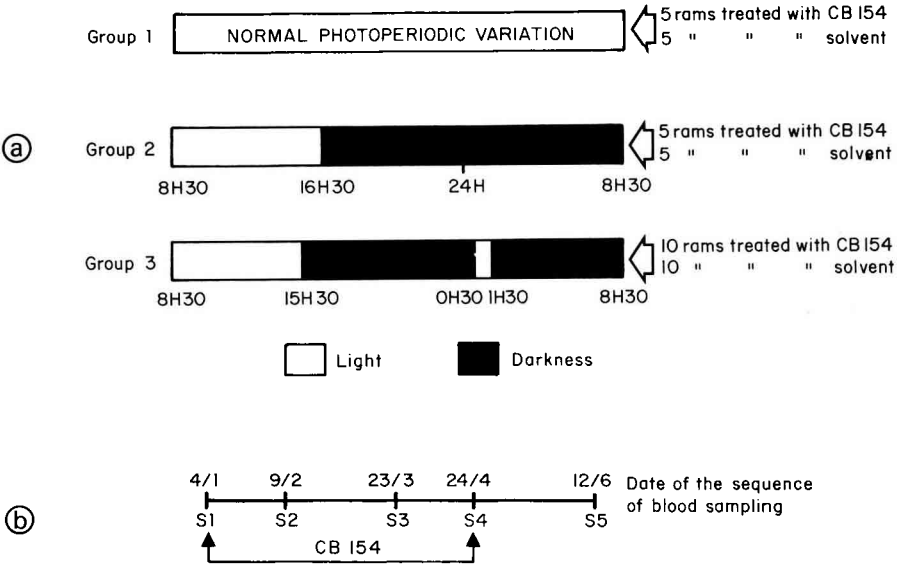

FIG. 1. — a— *Light schedules of different groups of rams.*
b — *Duration of CB 154 treatment and dates of hourly blood sampling.*

Hormone radioimmunoassay. — Plasma prolactin (Kann, 1971), FSH (Blanc and Poirier, 1979) and LH (Pelletier *et al.,* 1968) were measured by double antibody radioimmunoassay using NIH-P-S_6, HG-FSH-225 (= 2,6 NIH-FSH-S_3) and LH-CNRS-M_3 (= 1,8 NIH-LH-S_1), respectively, as standards. Testosterone was measured by the method of Garnier *et al.* (1979) without extraction.

Expression of results.

Prl. — We evaluated the effects of the different light treatments and the CB 154 by the mean levels (ng/ml plasma ± SEM) of the 25 hourly samples taken at each blood sampling sequence (S_1, S_2, S_3, S_4, S_5). Student's t-test was used to compare the plasma hormone levels between untreated and treated rams.

LH and T. — The results were expressed as the mean number of pulses/24 h/ram. At least 95 p. 100 of the LH peaks and 98 p. 100 of the testosterone peaks can be detected with hourly sampling intervals (Terqui *et al.,* 1980). The Mann-Whitney test (1947) was used to compare the two treatment groups at the same time of sampling, and the sign test (Dixon and Mood, 1946) was used to compare two successive blood sampling sequences.

FSH. — Since the circadian variation in FSH concentrations is slight (Ravault *et al.,* 1980), the FSH was measured in single plasma samples collected weekly. The data for FSH concentration were analysed by split-plot analysis of variance.

Testicular diameter. — In addition to the same statistical analysis as for FSH, we used Student's t-test between two dates to see if the variation in testicular diameter was significant during and after CB 154 treatment.

Results.

Prolactin (table 1). — At the beginning of the experiment before any treatment, the mean daily Prl levels in the various treatment groups were the same. After one month of artificial light treatment, hyperprolactinaemia was observed in group 3 ($P < 0.001$), but it did not remain constant. Prl levels decreased in all groups treated with CB 154 ($P < 0.001$). When treatment was withdrawn, the Prl levels increased again. This was conspicuous in group 3 in which the level (137 ± 12 ng/ml) was similar to that of solvent-treated rams subjected to light pulses for one month (119 ± 6 ng/ml).

LH and T (fig. 2). — At the beginning of the experiment (S_1), the number of peaks was similar in all groups ($P > 0.01$). In groups 1 and 2 during the period of CB 154 injection, there was no significant difference between untreated and treated rams. But when the treatment was withdrawn, the number of peaks increased significantly in group 2 ($P < 0.01$; ③ and ④, fig. 2). This pattern in group 3 was different ; while the number of LH and T peaks increased in solvent-treated animals, it did not augment in CB 154-treated ones (⑤ and ⑥, fig. 2). The difference between the treated and untreated rams was significant ($P < 0.01$) for the S_3 and S_4 sequences. As in group 2, at the end of CB 154 treatment, the number of peaks in treated animals increased significantly, reaching the same value as that in untreated animals.

TABLE 1

Plasma concentrations of Prl (ng/ml ± SEM) *in control* (C) *and CB 154-treated rams subjected to different light schedules*

		S_1 ↓	S_2	S_3	S_4 ↓	S_5
Group 1	C	35 ± 10	31 ± 8	27 ± 5	54 ± 8	65 ± 3[b]
	CB 154	42 ± 3	2.4 ± 0.1[ac]	1.5 ± 0.8[ac]	< 1[ac]	71 ± 5
Group 2	C	42 ± 4	52 ± 3	64 ± 3	35 ± 11	44 ± 14
	CB 154	36 ± 5	3.7 ± 1.2[ac]	1.4 ± 0.1[ac]	< 1[ac]	21 ± 5
Group 3	C	35 ± 3	119 ± 6[a]	46 ± 4	39 ± 5	28 ± 3
	CB 154	34 ± 4	1.7 ± 0.9[ac]	1.8 ± 0.1[ac]	< 1[ac]	137 ± 12[ac]

S_1, S_2, S_3, S_4, S_5 are the dates of blood sampling (see Material and methods). The arrows indicate the beginning and end of CB 154 treatment.
Superscript a : $P < 0.001$; comparison with solvent-treated rams during S_1.
Superscript b : $P < 0.05$; comparison with solvent-treated ram during S_1.
Superscript c : $P < 0.001$; comparison with solvent-treated rams during S_1.

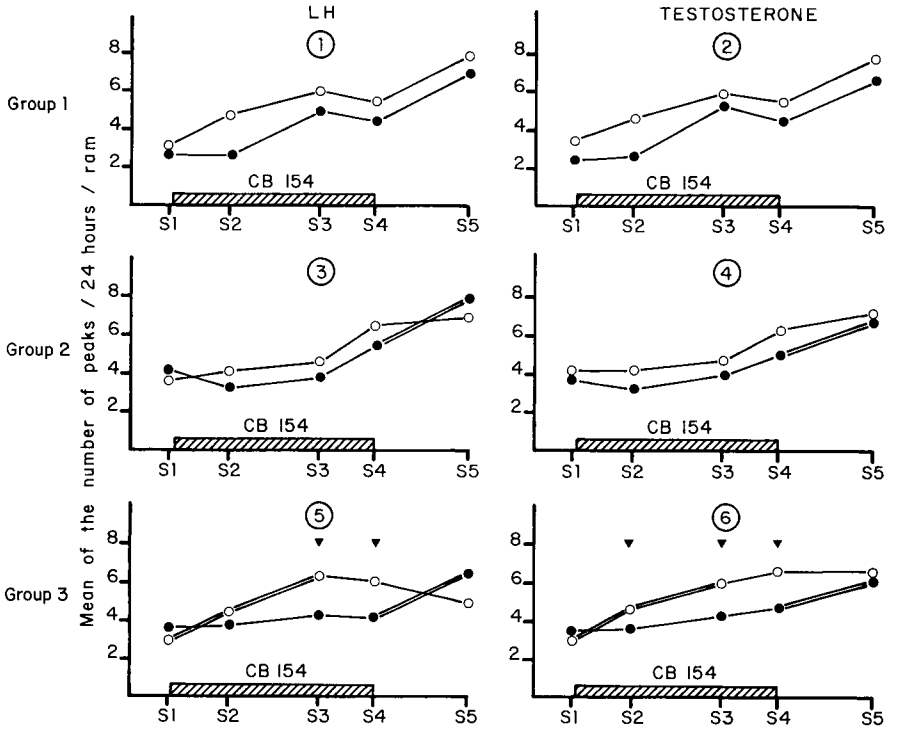

FIG. 2. — *Number of peaks of LH and T/24 h/ram in the three groups.*
o——o untreated (solvent), •——• CB 154-treated, •═══• Significant difference between two sequences, o═══o (sign-test ; $P < 0.01$).
▼ Significant difference between solvent-treated and CB 154-treated group in the same sequence (Mann-Whitney test ; $P < 0.01$).

FSH (fig. 3). — Compared with solvent-treatment rams ($P < 0.01$), the CB 154 treatment significantly increased plasma FSH levels in group 1. But in groups 2 and 3, CB 154 had no significant effect and did not prevent the increase in FSH levels at the end of the treatment ($P > 0.01$).

Testicular diameter. — Table 2, presenting testicular diameter at the end of the CB 154 treatment (24/4), shows that testicular growth was significantly reduced in group 3 during CB 154 treatment.

After that treatment was withdrawn, testicular growth was delayed only in group 3, and the control values for testicular diameter were not reached until 3 weeks.

Discussion.

The treatments used were effective in increasing and decreasing prolactin levels. In an earlier experiment, Ravault (1976) showed that normal variations of daylight ratio influenced the Prl level in untreated animals ; in the present experi-

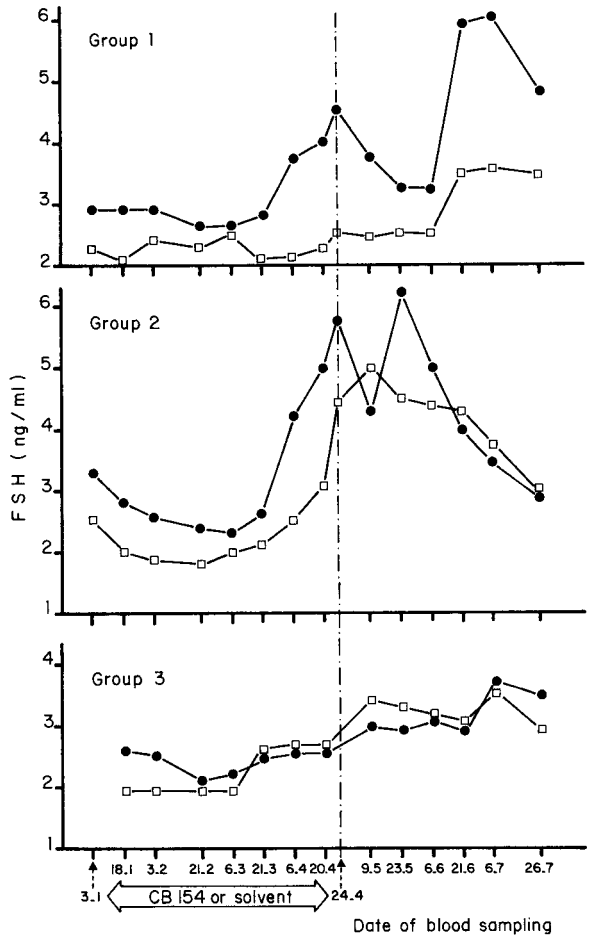

Fig. 3. — *Plasma concentration of FSH* (ng/ml) *in control* (▫——▫) *and* CB 154*-treated* (•——•) *rams subjected to different light schedules.*

TABLE 2

Testicular diameter (in cm) *at the end of CB 154* (24/4) *or solvent treatment in rams subjected to different light regimens*

	Control	CB 154	
Group 1	5.51 ± 0.10	5.64 ± 0.45	NS
Group 2	5.82 ± 0.11	5.58 ± 0.53	NS
Group 3	6.05 ± 0.25	5.60 ± 0.07	$P < 0.01$

ment, the Prl level increased from 35 ± 10 ng/ml in January to 65 ± 3 ng/ml in June (group 1). In rams subjected daily to 8 h of light, the Prl level remained constant (Ravault and Ortavant, 1977). Light pulses given daily 16 h after dawn have been shown to increase the level of Prl in rams (Ravault and Ortavant, 1977). In the present experiment, after one month of such light pulses the Prl level increased from 35 ± 3 ng/ml to 119 ± 6 ng/ml ($P > 0.001$), but then decreased again after 2 months of light treatment. In all CB 154-treated animals, prolactin concentration decreased to very low levels (1.5 ng/ml) and when treatment was withdrawn, it then increased to the same level as in solvent-treated rams (groups 1 and 2) or higher (group 3), as described by Barenton and Pelletier (1980).

In group 1, the number of peaks of LH and T/24 h/ram tended to increase from S_1 to S_5 in accordance with the observation of an increase between January and June (Terqui *et al.*, 1980 ; Pelletier *et al.*, personal data). The failure of this trend to reach statistical significance in the present experiment may be due to the small number of animals used.

In group 2 the number of LH and T peaks was not affected by CB 154 during the treatment period, agreeing with the results of Land *et al.* (1980). However, after treatment had finished, the number of peaks rose significantly.

In group 3 the mean frequency of the LH and T peaks was constant during S_2, S_3, and S_4 in CB 154-treated rams. But at the end of this treatment, the mean frequency of the LH and T peaks was elevated. In untreated rams, the mean frequency increased during S_2 and S_3, and in S_3 and S_4 it was higher than in CB 154-treated rams. During S_2, S_3 and S_4, the prolactin levels were very low in the CB 154-treated group. As the light regimen was identical for the two groups, CB 154 seems to have prevented the increase of the mean frequency of the LH and T peaks in treated rams. Furthermore, after the end of CB 154 treatment, this mean frequency increased in the CB 154 group as Prl rose. In comparison, it appears that the increase of Prl in untreated rams during S_2 and S_3 might explain the increase of the mean frequency of the LH and T pulses.

Under natural photoperiod (group 1), plasma FSH levels were higher in CB 154-treated rams than in solvent-treated ones ; this result supports that of Barenton *et al.* (1982) studying Romanov rams treated with CB 154 in summer. Similarly, Seki *et al.* (1974) and Nader *et al.* (1975) showed that CB 154 increased FSH secretion in women ; however, CB 154 did not increase this secretion in ewes (Land *et al.*, 1980).

Are these modifications in LH and FSH secretion due to the direct action of CB 154 on the hypothalamo-hypophysial axis, or are they the secondary effects of variations in prolactin level ? The frequency of LH pulses (or LH levels) decreases in lactating women (Bohnet and Schneider, 1977), lactating ewes (Schirar, 1977) and hyperthermic ewes (Hill *et al.*, 1980), all of which are hyperprolactinaemic. CB 154 appears to be a direct agonist of dopamine (Corrodi *et al.*, 1973), and several authors have shown that in various experimental conditions, dopamine inhibits the release of LH-RH (Uemura and Kobayashi, 1971 ; Gallo and Drouva, 1979) and thus of LH. In hypoprolactinaemic rats (treated with CB 154) the hypothalamic LH-RH level was not changed (Harper *et al.*, 1976 ;

Skett *et al.*, 1978 ; Vermes and Telegdy, 1978 ; Caraty and Martinat, 1979) ; this might explain why the number of LH and T pulses is not different between untreated and treated rams in groups 1 and 2. Prolactin could conceivably play a role in mediating the photoperiodic effect on the LH and T secretion that occurred during S_4 and S_5 in CB 154-treated rams and during S_2 and S_3 in solvent-treated rams of group 3. However, this seems unlikely since Prl injection over a short time did not change the levels of LH and FSH in the rams. Furthermore, other experiments in the ram (Blanc, unpublished data) show that FSH secretion is not necessarily under the influence of LH-RH.

In group 3, the CB 154 treatment delayed testicular growth which depends on FSH, LH and T (Courot and Ortavant, 1981). But the results are only evident in group 3 in which the FSH levels were identical in treated and untreated animals. Since it seems that CB 154 neither decreases FSH secretion nor changes the number of LH receptors in the ram testis (Barenton and Pelletier, 1980), the delay in testicular growth is probably due to the decrease of LH and T.

The particularly clear results in group 3 (hyperprolactinaemic rams) indicate that the number of LH and T pulses could be Prl-dependent.

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Résumé.— *Influence de la 2-Br-α-ergocryptine (CB 154) sur la sécrétion de prolactine, LH, FSH et testostérone et sur la croissance testiculaire chez des béliers soumis à différents régimes lumineux.*

L'influence de la 2-Br-α-ergocryptine sur la sécrétion des gonadotrophines et sur la fonction testiculaire a été étudiée chez des béliers soumis soit aux variations normales de la durée du jour, soit à un régime lumineux adéquat provoquant une hyperprolactinémie. Dans le groupe hyperprolactinémique, le traitement par le CB 154 produit une baisse du nombre moyen des pulses de LH et T par rapport à celui des animaux non traités. Après l'arrêt du traitement par le CB 154, la fréquence des pulses de LH et T réaugmente chez les animaux soumis à 8 heures de lumière ou rendus hyperprolactinémiques. Les concentrations plasmatiques de FSH augmentent pendant le traitement par le CB 154 uniquement chez les animaux soumis aux variations normales de la durée du jour. La croissance testiculaire est retardée chez les animaux traités par le CB 154 et rendus hyperprolactinémiques.

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