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THE EFFECT OF 2-Br-α-ERGOCRYPTINE ON THE HEPATIC STEROID METABOLISM AND SERUM PITUITARY HORMONE LEVELS IN NORMAL RATS AND RATS WITH AN ECTOPIC PITUITARY

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Abstract—The effect of a dopaminergic agonist, CB-154 (2-Br-α-ergocryptine), on the hepatic steroid metabolism in normal male and female rats, and in hypophysectomized male animals bearing an implanted pituitary under the kidney capsule, has been investigated. The serum levels of the four pituitary hormones LH, FSH, prolactin and growth hormone, were also measured. In normal animals, CB-154 reduced the serum level of prolactin without significantly affecting levels of LH, FSH or growth hormone and without masculinizing hepatic steroid metabolism of female rats or feminizing hepatic steroid metabolism of male rats. Implantation of a pituitary gland from age-matched female rats in hypophysectomized male rats caused an increase in prolactin and growth hormone levels in serum and a shift towards a more feminine type of steroid metabolism. Treatment with CB-154 reduced the prolactin level without affecting LH, FSH or growth hormone levels and without masculinizing hepatic steroid metabolism. In conclusion, the drug, CB-154, causes a marked and selective decrease in circulating prolactin levels without affecting the sex differentiation of hepatic steroid metabolism thus indicating that these two parameters are not related. A certain correlation between growth hormone levels and degree of femininity of steroid metabolism was seen but the significance of this is debatable.

In recent years, attention has been focused on the control of the sexual differences of hepatic steroid metabolism by the pituitary gland [1–10] and a novel pituitary hormone has been postulated to be involved in this regulation using data obtained from *in vitro* experiments [7, 11]. It has, however, proved difficult to rule out the known pituitary hormones as effectors in this regulatory mechanism using *in vivo* techniques such as injection of the various hormones mainly due to the short half-life in serum of these proteins [12–15].

Consequently another approach was necessary, namely the use of drugs which selectively affect the secretion of one of the pituitary hormones. We have employed the dopaminergic agonist, 2-Br-α-ergocryptine (CB-154) [16, 17] as a selective inhibitor of prolactin secretion, and studied the effects of this drug on hepatic steroid metabolism and pituitary hormone secretion from both pituitary glands *in situ* and glands implanted under the kidney capsule of hypophysectomized male rats.

Implantation of a pituitary gland under the kidney capsule of male rats is known to feminize the pattern of hepatic steroid metabolism [3, 5, 6, 18] and increase the serum level of prolactin and growth hormone [18, 19], but the functional relationship between these observations is not clear.

In this paper we have attempted to answer the question—what effect does CB-154 have on sex differentiation of hepatic steroid metabolism and how does this correlate with its effect on serum levels of pituitary hormones?

MATERIALS AND METHODS

Animals of the Sprague–Dawley strain (Anticimex, Stockholm, Sweden) were used throughout the study. The rats were received at 6–7 weeks old and housed in a light- and temperature-controlled animal room (lights on 06.00–20.00 at 23°C ± 1°). All animals were given food and water *ad libitum* except after hypophysectomy when water was replaced by 5% (w/v) glucose–0.9% (w/v) sodium chloride solution. In experiment 1, male and female rats (7 weeks old) were i.m. injected twice daily with 20 μg of CB-154 in 100 μl of 70% (v/v) ethanol in water for 1 week prior to incubation. Control animals were injected similarly with solvent only. In experiment 2, hypophysectomy was carried out on rats, 6 weeks of age, by the parapharyngeal route under ether anaesthesia 2 weeks prior to incubation. One week before incubation the animals were divided into three groups and a fresh pituitary from an age-matched female rat was inserted under the renal capsule of the animals in two of the groups; of these groups one was treated with two daily doses of 20 μg CB-154 for 7 days whilst the other group received only solvent (100 μl 70% (v/v) ethanol in water) during this period. A third experiment, whereby CB-154 was injected into hypophysectomized male animals in the same manner as described above, was performed to ascertain the effects on hepatic steroid metabolism of CB-154 which are not mediated by the pituitary gland.

On the morning of incubation, a blood sample was taken from each animal. The blood was allowed to coagulate at 4° and the serum collected and stored at 20° until analyzed for pituitary hormones. After

blood sampling the animals were killed by cervical dislocation and the livers quickly excised and put in ice-cold Bucher medium [20]. The appearance of the pituitary graft was checked in the respective rats and only rats showing acceptable transplantations (by gross morphological examination) were used. The liver was homogenized and separated into cytosol and microsomal fractions by fractional centrifugation as described previously. The two fractions were then incubated with 4-[4-^{14}C]androstene-3,17-dione (New England Nuclear, sp. act. = 57.5 mCi.mmole^{-1}) (cytosol and microsomes) and 5α-[4-^{14}C]-androstane-3α,17β-diol (microsomes only) (prepared as already described [21]) in the same manner as in earlier publications [21, 22]. The incubation products have been identified during previous studies [21, 22].

Protein determinations were performed according to the method of Lowry *et al.* [23] and enzyme activities expressed as nmoles of product formed/min/mg protein.

Radioimmunoassay for LH, FSH, growth hormone and prolactin was performed using the kits supplied by the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism and Digestive Diseases to whom we are grateful for this service. A modification of the assay technique recommended by the NIAMDD was used as previously described [24]. Means and S.D. were calculated in all experiments and statistical analysis was performed using Student's '*t*' test and, in the case of experiment 2, Duncan's multiple range test. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The following enzyme activities could be calculated from the amount of metabolites formed in the incubations: 5α-reductase, 6β-, 7α- and 16α-hydroxylase and 17β-hydroxysteroid oxidoreductase (in microsomal fraction, active on 4-androstene-3,17-dione), 5β-reductase (in cytosol fraction, active on 4-androstene-3,17-dione) and the 2α-, 2β-, 7α- and 18-hydroxylase (active on 5α-androstane-3α, 17β-diol) activities.

As can be seen from experiment 1 (Table 1), injection of CB-154 into normal male and female animals had certain effects on liver steroid metabolism—an increase of the 5α-reductase and 6β-hydroxylase ($P < 0.05$) (active on 4-androstene-3, 17-dione) and a decrease of the 7α-hydroxylase activities (active on 5α-androstane-3α,17β-diol) ($P < 0.05$) in the female rat and a decrease in the 16α-hydroxylase (active on 4-androstene-3, 17-dione) ($P < 0.01$) and the 2α- and 2β-hydroxylase activities (active on 5α-androstane-3α,17β-diol) ($P < 0.05$) in the male rat. When comparing these results with those obtained for the normal animals, the changes seen could not be interpreted as significant tendencies towards a more masculine pattern in the females or a more feminine pattern in the male animals after CB-154 treatment. Of the pituitary hormones studied only prolactin was significantly affected in both sexes by CB-154 treatment (a decrease of approximately 75 per cent in serum concentration in both sexes). This is an expected effect of CB-154 considering its effect on the dopaminergic nerve system in the brain [16, 17]

Table 1. The effect of CB-154 on the hepatic steroid metabolism of normal male and female rats

	Female		Male	
Enzyme activity	Saline	CB-154	Saline	CB-154
Active on 4-androstene-3,17-dione				
5α-reductase	26.1 ± 4.5	43.9 ± 9.9*	12.4 ± 3.4	14.9 ± 5.2
16α-hydroxylase	N.D.	N.D.	1.22 ± 0.12	0.60 ± 0.20†
6β-hydroxylase	0.26 ± 0.01	0.52 ± 0.17*	0.91 ± 0.48	0.87 ± 0.31
7α-hydroxylase	0.48 ± 0.04	0.65 ± 0.17	0.48 ± 0.24	0.60 ± 0.05
17β-HSR	0.54 ± 0.05	0.46 ± 0.06	1.10 ± 0.09	1.02 ± 0.20
5β-reductase	0.17 ± 0.03	0.22 ± 0.03	1.16 ± 0.28	1.01 ± 0.21
Active on 5α-androstane-3α,17β-diol				
2α-hydroxylase	N.D.	N.D.	0.28 ± 0.08	0.12 ± 0.03*
2β-hydroxylase	N.D.	N.D.	0.17 ± 0.04	0.08 ± 0.02*
7α-hydroxylase	0.85 ± 0.08	0.52 ± 0.09*	0.37 ± 0.04	0.32 ± 0.02
18-hydroxylase	N.D.	N.D.	0.43 ± 0.10	0.22 ± 0.14
Hormone				
LH	109 ± 26	143 ± 31	132 ± 21	107 ± 12
FSH	213 ± 57	358 ± 107	360 ± 84	322 ± 36
Prolactin	52 ± 5	12 ± 2‡	56 ± 31	14 ± 2*
Growth hormone	23 ± 16	21 ± 20	9 ± 2	12 ± 9

In vitro activities of the enzymes present in microsomal and cytosol fractions of the liver were measured using 4-[4-^{14}C]androstene-3,17-dione and 5α-[4-^{14}C]androstane-3α,17β-diol as substrates. All activities were measured in the microsomes except for the 5β-reductase. Enzyme activities are expressed as nmoles of product formed min^{-1} mg protein^{-1} (mean ± 1 S.D., $n = 4$). Hormone concentrations are expressed as μg l^{-1} (mean ± 1 S.D., $n = 4$).
*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ as compared to appropriate control value.
17β-HSR = 17β-hydroxysteroid oxidoreductase.
N.D. = not detected (< 0.05 nmoles product min^{-1} mg protein^{-1}).

Table 2. The effect of CB-154 on the hepatic steroid metabolism of hypophysectomized rats bearing an implanted pituitary from a female rat under the kidney capsule

Enzyme activity	Hypox ♂	Hypox ♂ + PT§	Hypox ♂ + PT§ + CB-154
Active on 4-androstene-3,17-dione			
5α-reductase	2.6 ± 0.4	18.9 ± 4.7‡	15.7 ± 3.1‡
16α-hydroxylase	1.26 ± 0.07	0.43 ± 0.15‡	0.35 ± 0.05‡
6β-hydroxylase	1.63 ± 0.30	0.46 ± 0.21†	0.06 ± 0.20†
7α-hydroxylase	0.52 ± 0.03	0.48 ± 0.13	0.47 ± 0.09
17β-HSR	2.02 ± 0.38	1.46 ± 0.37	1.13 ± 0.47*
5β-reductase	0.47 ± 0.09	0.20 ± 0.12*	0.27 ± 0.17
Active on 5α-androstane-3α, 17β-diol			
2α-hydroxylase	0.12 ± 0.08	N.D.*	N.D.*
2β-hydroxylase	0.07 ± 0.03	N.D.*	N.D.*
7α-hydroxylase	0.50 ± 0.09	0.63 ± 0.14	0.66 ± 0.18
18-hydroxylase	0.65 ± 0.18	0.23 ± 0.09*	0.42 ± 0.12
Hormone			
LH	25	25	25
FSH	100	100	100
Prolactin	7 ± 2§§	88 ± 25†	9 ± 4§§
Growth hormone	17 ± 5	28 ± 5*	30 ± 5*

§ No significant differences were found in enzyme activities between the groups hypox ♂ + PT and hypox ♂ + PT + CB-154 according to Student's '*t*' test.

§§ Prolactin values in hypox ♂ and hypox ♂ + PT + CB-154 were not significantly different ($P > 0.05$) according to Duncan's multiple range test.

For further information, see Table 1. Values which are commonly underlined are not significantly different from each other ($P > 0.05$) according to Duncan's multiple range test.

Hypox = hypophysectomized.

PT = bearing pituitary implant.

and the apparent connection between this nerve system and the control of prolactin secretion [25, 26].

In experiment 2 (Table 2) the influence of CB-154 on the effect of implantation of an adult-derived pituitary gland on hepatic steroid metabolism in the hypophysectomized adult male rat was investigated.

The hypophysectomized animals showed a similar pattern of steroid metabolism as the control male animals in experiment 1 except for a decreased activity of the 5α-reductase. Although the two experiments are not directly comparable, these data confirm that the pituitary gland in the male does not play a major role in the control of hepatic steroid metabolism [2, 4–6]. Implantation of a female-derived pituitary gland under the kidney capsule of hypophysectomized male rats caused a marked change in the metabolic pattern. There was a greater than 7-fold increase in 5α-reductase activity ($P < 0.001$) and significant decreases in the 6β- and 16α-hydroxylase (active on 4-androstene-3, 17-dione; $P < 0.01$ and < 0.001, respectively), the 5β-reductase ($P < 0.05$) and the 2α-, 2β- and 18-hydroxylase activities (active on 5α-androstane-3α, 17β-diol; $P < 0.05$ in each case). All of these changes are indicative of a move towards a more feminine type of metabolism. This effect of an implanted pituitary has previously been demonstrated in male rats [5, 18]. According to Student's '*t*' test, there were no significant differences between the two groups bearing pituitary grafts. Statistical analysis using Duncan's multiple range test indicated that CB-154 further decreased the 16α-hydroxylase and 17β-hydroxysteroid oxidoreductase activities and increased the 6β-hydroxylase (active on 4-androstene-3,17-dione) and 18-hydroxylase (active on 5α-androstane-3α,17β-diol) activities. CB-154 treatment of male, hypophysectomized rats (experiment 3, Table 3) caused an increase of 5α-reductase activity without concomitant changes in any of the other enzymes studied. This effect is not seen in the presence of ectopic pituitary tissue probably due to the already raised level of 5α-reductase caused by the implanted pituitary gland. These data indicate that only the 5α-reductase activity is affected by CB-154 in the absence of an intact pituitary and all other effects would seem to be indirect.

Pituitary hormone levels were low in the hypophysectomized animals (Table 2) as would be expected if complete hypophysectomy had been performed. LH and FSH were below the level of detection (< 25 and < 100 μg/l, respectively), while prolactin levels were 7 ± 2 μg/l and growth hormone levels 17 ± 5 μg/l. The comparatively high growth hormone level is possibly due to the presence of an extra-pituitary source of the hormone. Implantation of a pituitary gland under the kidney capsule results in increases in the levels of prolactin (12-fold increase; $P < 0.01$) and growth hormone (165 per cent increase; $P < 0.05$) but no detectable LH or FSH. These results are in agreement with previous publications on the production of hormones by an ectopic pituitary gland [19]. Treatment of the animals implanted with a pituitary gland with CB-154 had no significant effect except for a decrease in the

Table 3. The effect of CB-154 on the hepatic steroid metabolism of hypophysectomized male rats

Enzyme activity	Hypox ♂	Hypox ♂ + CB-154
Active on 4-androstene-3,17-dione		
5α-reductase	0.85 ± 0.08	1.34 ± 0.12*
16α-hydroxylase	0.77 ± 0.22	0.70 ± 0.13
6β-hydroxylase	0.80 ± 0.08	1.00 ± 0.08
7α-hydroxylase	0.50 ± 0.09	0.60 ± 0.08
17β-HSR	0.62 ± 0.03	0.69 ± 0.05

* For further information see Tables 1 and 2.

prolactin level ($P < 0.01$) (Table 2) which is expected for this compound [16, 17]. It thus appears that CB-154 has a direct action on the implanted pituitary and reduces the prolactin secretion of the implant without affecting growth hormone secretion or hepatic steroid metabolism. The feminine type of hepatic steroid metabolism appeared to be positively correlated to the serum level of growth hormone. Tests on growth hormone using an *in vitro* assay for compounds causing feminization of steroid metabolism [11] have, however, proved negative. The relationship between growth hormone levels and feminine hepatic steroid metabolism may be coincidental rather than causal in this case although growth hormone has been implicated in the control of hepatic steroid [10] and drug metabolism [27].

In summary, the dopaminergic agonist, 2-Br-α-ergocryptine (CB-154), has a marked effect on the circulating level of prolactin released from the pituitary *in situ* or under the kidney capsule in the rat while not influencing the growth hormone level or sex differentiation of hepatic steroid metabolism. There is a positive correlation between growth hormone levels and degree of femininity of hepatic steroid metabolism but insufficient data are available to conclude that this is a causal relationship.

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